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HEREDITY VOLUME 6 PART 2 AUGUST 1952

THE HISTORY OF THE GARDEN NARCISSI

ANN P. WYLIE
John Innes Horticultural Institution, Hertford

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1. INTRODUCTION

THE object of the present study is to discover how far the study of chromosomes and a knowledge of hybridisation and documentary history can be used to reveal the origin and means of improvement of the garden daffodils. Previous cytological studies of the genus *Narcissus* have been made, principally by Fernandes (1934-1951) and Nagao (1929, 1933), both of whom considered the wild species for the most part. As a result of their labours, the cytological picture of the genus is now well established. There are two basic numbers: 7 is found in all the species except *N. tazetta* and the closely related *N. elegans* and *N. broussonetii*, in which it is 10 or the derived 11. Polyploidy is rare in the wild with the exception of *N. bulbocodium*. Most of the apparently wild polyploids reported in the other species are not certainly known to be native in the places where they were collected and are probably escapes from cultivation.

The genus is confined to the temperate Old World, with its headquarters in the Iberian peninsula. The northern limit is England, the southern the Canary Islands, while Asia Minor and Syria probably represent the easterly extremes of distribution. *N. tazetta* in its more easterly stations of Persia, Cashmir, China and Japan is doubtless subspontaneous only (Fernandes, 1951). Members of the genus have been cultivated for centuries and horticultural varieties have been created at an increasing rate since 1850, when a start was made with deliberate hybridisation. To-day there are over 12,000 named varieties. A list of the chromosomes numbers of a representative selection of these garden *Narcissi* has been published (Janaki-Ammal and Wylie, 1949).

2. MATERIALS AND METHODS

Bulbs and flowers of *Narcissi* were given me by the following, to whom I am most grateful: Col. F. C. Stern, Goring-by-Sea, Sussex; the Director and Dr E. K. Janaki-Ammal, R.H.S. Gardens, Wisley, Surrey; and W. O. Backhouse, Sutton Court, Hereford. Bulbs were also obtained from Messrs Barr and Son, W. Blom and A. Gray.

Somatic counts were made from root-tips, where possible, pretreated with mono-

bromonaphthalene (O'Mara, 1948), to which was added ·01% of a wetting agent, sulphonate of lorol, to assist in penetration and shorten the time necessary for pretreatment. The root-tips were then fixed in acetic alcohol (figs. 1, 5*b* and *c*, 9*a* and *b*, 10*b* and 11) or 2BE (figs. 2, 5*a* and *d*, 6, 9*c*, and 10*a*, *c* and *d*) and prepared as Feulgen squashes in the usual way. Where roots were not available, a useful source of mitoses proved to be nucellar and integumentary tissue in ovules from recently opened flowers. Strings of ovules were dissected out of the ovaries, pretreated and fixed as for root-tips and prepared as orcein or lacmoid squashes. Pollen grain divisions were obtained in aceto-carmine squashes of anthers fixed in acetic alcohol (figs. 8, 12) or else from smears fixed in 2BE and stained in Feulgen (fig. 7).

3. NARCISSUS SPECIES

(i) *General*

In order to give some idea of the sources of the garden narcissi of to-day, a brief survey of the species in the genus will be made, indicating the characters they have contributed to the various garden groups and the available cytological data.

As Fernandes (1951) has shown, the use of the basic chromosome number and breeding behaviour in defining the main species provides a far more natural classification than any of the many schemes previously proposed (e.g. Baker, 1888). The major subdivision is into the large group with 7 as the basic number and the smaller one of *N. tazetta* and relatives in which it is 10 or 11. The former includes the whole of Baker's Magni- and Medio-coronatae and part of his Parvicoronatae and the latter the remainder of the Parvicoronatae.

In Table 1 the more important species are divided into groups according to their chief diagnostic characters. Brief notes on these sexual species are given below.

TABLE 1

Principal characters of Narcissus *species*

<table>
<tr><th rowspan="2">Corona</th><th rowspan="2">Flowers per Spathe</th><th colspan="2">Leaves</th><th rowspan="2"></th></tr>
<tr><th>Flat or linear</th><th>Cylindrical</th></tr>
<tr><td rowspan="2">Long</td><td rowspan="2">one</td><td colspan="2">$x=7$</td><td rowspan="4">Not fragrant</td></tr>
<tr><td>pseudonarcissus
cyclamineus</td><td>bulbocodium
(2x, 4x, 6x)</td></tr>
<tr><td>Medium</td><td>two plus</td><td colspan="2">triandrus</td></tr>
<tr><td rowspan="3">Short</td><td rowspan="2">one</td><td colspan="2">watieri</td></tr>
<tr><td>poeticus</td><td>rupicola
atlanticus</td><td rowspan="3">Fragrant</td></tr>
<tr><td rowspan="2">two plus</td><td>$x=10, 11$</td><td rowspan="2">calcicola
scaberulus
fernandesii
juncifolius
gaditanus
jonquilla
viridiflorus
serotinus</td></tr>
<tr><td>tazetta
elegans
broussonetii</td></tr>
</table>

(ii) *Sexual Species*

1. *N. pseudonarcissus* L., the common daffodil. Great variation is found in size and flower colour throughout its range and led Pugsley (1933) to split it into 26 species. However, as A. and R. Fernandes (1946) point out, there is no justification for this from the cytological data. Practically all are diploids. The cultivated varieties *hispanicus* (=*maximus*) and *tortuosus* (Fernandes, A. and R., *l.c.*) are triploids, and *bicolor* is reported by the Fernandes as a tetraploid. Polyploids have arisen or at least have been preserved in cultivation. B chromosomes in several varieties (section iv).

2. *N. cyclamineus* DC. is distinguished from the closely related *pseudonarcissus* by the longer corona and sharply reflexed perianth segments, a character which is dominant in crosses with other species and garden varieties. It has a very limited distribution in Portugal. Like *pseudonarcissus*, it is a diploid (A. and R. Fernandes, 1946) and cytologically indistinguishable from that species (fig. 1*b*). It is only recently that it has been used in hybridising with garden narcissi.

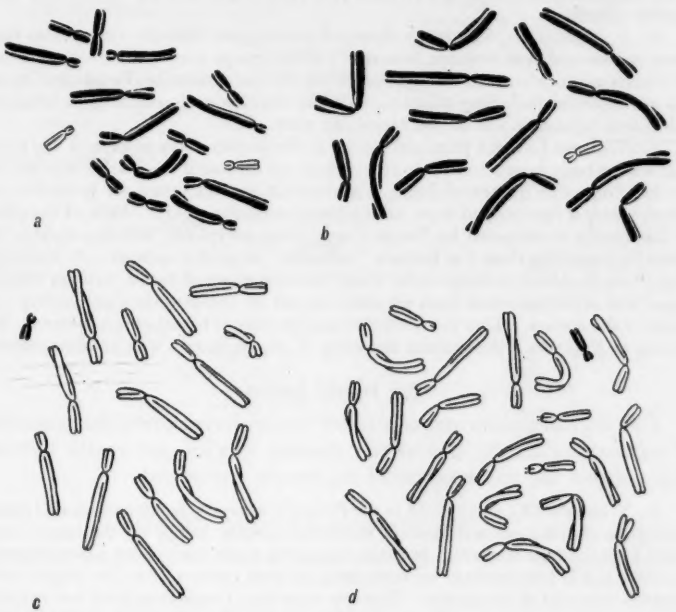

FIG. 1.—Supernumerary chromosomes in root tip mitoses of species and hybrids.
(*a*) *N. bulbocodium citrinum*, $2n=14+2$ heterochromatic B's. (*b*) *N. cyclamineus*, $2n=14+1$ B. (*c*) *N. X bernardi*, $2n=14+1$ B. (*d*) the *cyclamineus* hybrid "Beryl" with $2n=21+1$ B.
(*a*) and (*b*) × 2,200: (*c*) and (*d*) × 1,700.

3. *N. bulbocodium* L. shows the maximum development of the corona in the genus. It is highly variable in size, colour and form and is the only species in which polyploidy is important. Fernandes (1947) has reported diploid to hexaploid varieties. I found 29 chromosomes in the recently introduced variety *romieuxii* from the Middle Atlas mountains, a previously unreported number for the species. The extra chromosome was an additional representative of one of the normal complement. This species has been little used so far in breeding garden narcissi, except for a few recent

hybrids with such dwarf species as *N. triandrus* and members of the *jonquilla* group.

4. *N. triandrus* L. is one of the group with more than one flower per scape (table 1) and has a moderately developed corona. Again all the varieties are diploids (Fernandes, 1949). This species, too, has been used only recently for crossing with garden narcissi.

5. *N. poeticus* L. is second only to *pseudonarcissus* in its importance as an ancestor of the garden narcissi. It is widely distributed throughout S. and S.E. Europe and naturalised in many places elsewhere. It, too, is a variable species and has been split into 9 by Pugsley (1915). All forms so far examined are diploid, with the exception of the variety *recurvus* which I found to be triploid. No wild locality is known for this variety, however, (Pugsley, *l.c.*), and it is probably a garden escape like the polyploids in the *pseudonarcissus* complex. The main characters of this species are the one-flowered scape (table 1), the uniformly white perianth segments, the long perianth tube and the disc-like corona, which is either suffused with a red or orange pigment or has this colouring restricted to a narrow rim. This pigment is the ultimate source of all the brilliant red and orange shades found in the modern garden clones.

6. *N. jonquilla* L., with many flowered spathes and rush-like leaves, also has a short corona and very fragrant flowers. 7 allied species may also be included here, all dwarfs and of restricted distribution in the Iberian peninsula (Fernandes, 1939*c*). All are diploids, including *atlanticus*, with the exception of *viridiflorus*, a tetraploid found near Gibraltar and on the Moroccan coast.

7. *N. tazetta* L. is the principal species in the second main division of the genus, that with a basic number of 10 or 11. It is found all round the Mediterranean, but the localities often quoted of Persia, Cashmir, China and Japan are probably only places where it has escaped from cultivation (Fernandes, 1951). Most of the plants of this species investigated by Nagao (1933) were polyploids, which is further evidence for regarding these Far Eastern " varieties " as garden escapes. *N. broussonetii* Lag., from N. Africa, belongs to the white flowered group of *tazetta* varieties (Bowles, 1934) and is distinguished from all other species by the extreme rudimentary condition of the corona. Like the white flowered *tazettas*, it has 22 chromosomes. Also related to *N. tazetta* is the autumn flowering *N. elegans* Spach. with 20 chromosomes.

(iii) *Hybrid Species*

Cytological studies and controlled crosses have shown that a number of narcissi, originally considered distinct species, are really hybrids. Fig. 2 shows the relationships of the species concerned.

1. *N. bernardi* DC. occurs wild in the Pyrenees wherever *pseudonarcissus* and *poeticus* overlap in distribution and time of flowering (*poeticus* being, on the whole, some weeks later than the former). It varies according to the form of the parental species involved and is intermediate between them in such characters as the length of the perianth tube and of the corona. The one clone that I have examined was a diploid with 14 + 1B chromosomes (Fig. 1*c*). *N. bernardi* is the only fertile diploid hybrid species. Engleheart (1894) reports that among the progeny from *bernardi* selfed, forms were found which covered the whole range of variation from *pseudonarcissus abscissus* through *bernardi* to *poeticus*. *N. bernardi* is the wild counterpart of the garden hybrids formerly grouped under *N. incomparabilis* and now called the large-cupped narcissi.

2. *N. johnstonii* Pugs. was discovered in 1885 in Portugal and later in Spain. On morphological grounds this species was considered to be a natural cross of *triandrus* and *pseudonarcissus* and similar forms were raised by Engleheart (1890) from crossing the triploid trumpet daffodil " Emperor " with *triandrus*. The clones examined by Philip (1934), Fernandes (1945) and me were all triploids. Since the hybrid looks more like *pseudonarcissus* than *triandrus*, it is likely that 14 chromosomes were contributed by the former rather than the latter parent, probably through a diploid gamete.

3. *N. odorus* L. is presumably of ancient garden origin, since no wild locality is known for it. It is intermediate between *pseudonarcissus* and *jonquilla* and Herbert (1842) raised scarcely distinguishable plants by crossing these two species. Pereira (1940) found it to be a diploid in which chromosome types characteristic of the two supposed parents could be recognised. The very irregular meiosis that she found and its complete sterility give further evidence of its hybrid origin.

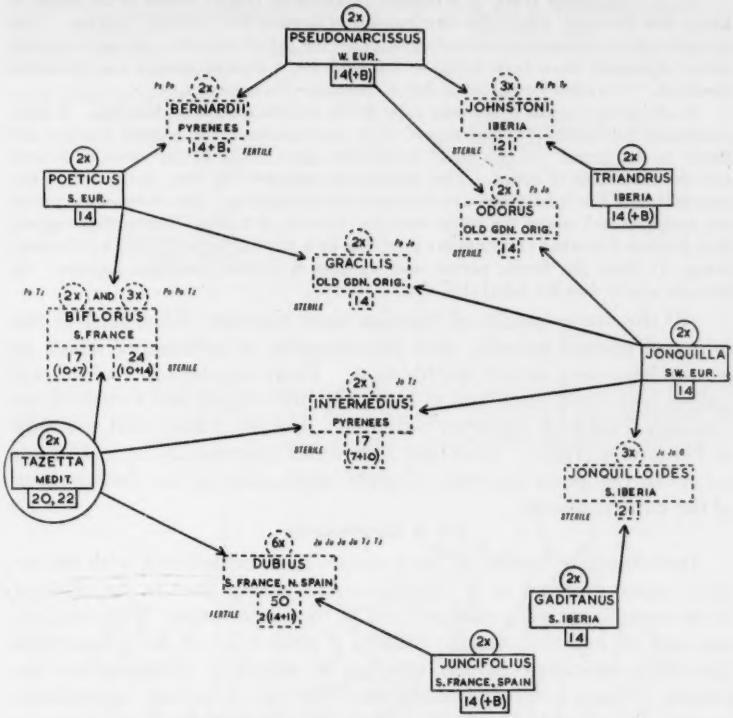

FIG. 2.—Diagram showing the natural interspecific hybrids which have been studied cytologically.

4. *N. gracilis* Sab. and its variety *tenuior* Curt. also has no wild locality known. It combines the characters of *jonquilla* and *poeticus* and Engleheart (1894) by crossing these two species produced quite typical specimens of *gracilis*. Pereira (1940) reports that *gracilis* is a diploid and I have found the same for *tenuior*. Lack of pairing at meiosis and complete sterility again demonstrate its hybridity.

5. *N. intermedius* Lois. is also supposed to have *jonquilla* as one parent. The other, on morphological grounds and as a result of Engleheart's crosses (1894), was considered to be *tazetta*. Nagao (1933) found it to have 17 chromosomes, with complete lack of pairing at meiosis and sterile pollen. It has been collected wild from the Pyrenees and is quite sterile.

6. *N. biflorus* Curt. is considered to be the result of crossing *tazetta* and *poeticus*. It has been long cultivated, but the cross has apparently also taken place in the wild in the south of France (Bowles, 1934). It is intermediate in appearance between

the supposed parents and was synthesised by Engleheart (1894). Nagao (1933) found it to have 24 chromosomes, which usually formed 7 bivalents and 10 univalents at meiosis. Its formula is thus PoPoTz. Fernandes (1934) reports a plant of this "species" with 17 chromosomes, which must then have arisen from the fusion of haploid gametes (PoTz). The two types are recognisable, as *biflorus* is reported as being sometimes much closer to *tazetta* than at others. It is again quite sterile.

7. *N. jonquilloides* Willk. is a triploid (Fernandes, 1939*a*), found in the south of Spain and Portugal, where the distributions of *jonquilla* and *gaditanus* overlap. The morphological and cytological findings support the JoJoG formula. As no tetraploid forms of *jonquilla* have been found in the wild yet, a diploid gamete was probably involved. Trivalent formation at MI is common (Fernandes, *l.c.*).

8. *N. dubius* Gouan is the only fully fertile hybrid species in *Narcissus*. I have confirmed Fernandes' (1937*a*) report of 50 chromosomes, the highest number yet found in the genus. It is a small *tazetta*-like plant found in the south of France and the N.E. coast of Spain. This distribution supports the idea, derived from the appearance of the hybrid and also chromosome morphology, that the parent species are *juncifolius* and one of the white varieties ($2n=22$) of *tazetta*. Fernandes suggests that it arose following chromosome doubling in a triploid hybrid with 25 chromosomes, 11 from the *tazetta* parent and 14 from a diploid *juncifolius* gamete. Its formula would then be JuJuJuJuTzTz.

All the major species of *Narcissus* have therefore taken part in the origin of natural hybrids, with the exception of *cyclamineus*, which as we saw has a very limited distribution. There are also some additional species which are suspected to have a hybrid origin, but for which no cytological information is yet available. Details of these will be found in Fernandes (1951). The ease with which interspecific hybridisation occurs in the genus has been of great importance in the development of the garden narcissi.

(iv) *B Chromosomes*

Individuals of several of these species have been found with one or more supernumerary or *B* chromosomes. These float in populations of the sexual species or may be fixed in the clonal ones. They may or may not be heterochromatic. Table 2 gives a list of the species and also their associated garden varieties in which *B* chromosomes are present. Since heterochromatin does not occur in any appreciable quantity in normal plants, it is easy to tell whether the *B* chromosome is heterochromatic or not.

In all the species and varieties which I have found to contain *B* chromosomes, this is easily recognised. It is about two-thirds the size of the trabant-bearing chromosome, normally the shortest in the complement, and it has a subterminal centromere (fig. 1). All the *B* chromosomes I have found are very similar and all euchromatic except for the two found in one plant of *N. bulbocodium citrinum* (fig. 1*a*). Two large chromocentres in the resting nuclei of this plant show that the *B*'s are heterochromatic. In other varieties of the same species, Fernandes (1947) has reported from 1 to 4 *B*'s, which may be either eu- or hetero-chromatic. The heterochromatic *B* found by Fernandes (1939*b*) in *N. juncifolius* differs from those in the other species, in being nearly as large as one of the normal long chromosomes.

The *B*'s found in the garden varieties listed in table 2 are all

TABLE 2

B chromosomes in Narcissus

Species or Variety	No. of B's	Authority
I Heterochromatic		
pseudonarcissus asturiensis .	1	A. and R. Fernandes 1946
juncifolius	1 (large)	Fernandes 1939*b*
bulbocodium . . .	1-4	Fernandes 1947
,, *citrinum* . .	2	A. P. W.
II Euchromatic		
pseudonarcissus asturiensis .	1, 2	A. P. W.
minor . .	1	Collins, in Pugsley 1933
pallidiflorus .	1	Philp 1934
pumilis	1	Collins *l.c.*
,,	1	A. P. W.
bernardi . . .	1	,,
Trumpet Daffodils		
" Empress " (3x) . .	1	,,
" Victoria " (3x) . .	1	,,
cyclamineus . . .	1	,,
cyclamineus hybrid " Beryl " .	1	,,
triandrus albus . .	1	,,
calcicola . . .	1, 2	,,

euchromatic and of the same type as those found in the related species and are presumably derived from them. The extra chromosome in " Empress " (fig. 3*a*) is therefore not a fourth representative of one of

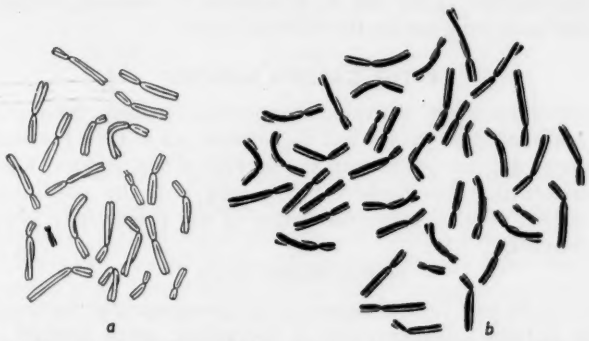

FIG. 3.—(*a*) Somatic chromosomes of the trumpet variety " Empress " with $2n=21+1$ B. (*b*) root tip mitosis in a trumpet seedling with 41 chromosomes. × 1,120.

the longest chromosomes of the normal complement, as reported by the Fernandes (1946). The triploid bicolor trumpet " Victoria " also has a euchromatic *B*. Again the extra chromosome is not one of the normal, long chromosomes, as Sikka (1940) suggests. Two other triploid trumpets (now perhaps extinct), " Grandis " and " Horsefieldii " have been reported to have 22 chromosomes, by Nagao (1929) and by Philp (1934). Here also perhaps the extra chromosome is a *B*.

The *cyclamineus* hybrid " Beryl " (fig. 1*d*) also has 22 chromosomes,

of which one is a *B* and similar to one found in a plant of *N. cyclamineus* (fig. 1*b*).

(v) *The Two Basic Numbers*

The chromosomes of the species with basic number 7 all have median or submedian centromeres (e.g. figs. 1, 6*b*) with minor differences only between the complements of the different species (Fernandes, 1951). But in *tazetta* and relatives with basic number 10 or 11, 8 of these chromosomes have subterminal centromeres (fig. 6*a*). As Fernandes (*l.c.*) has shown, 7 is almost certainly the original number for the genus and 10 the derived one. The latter could have arisen from 7 by fragmentation near the centromere, as in *Fritillaria* (Darlington, 1936).

I have examined material from Col. F. C. Stern of the closely related genus *Tapeinanthus*, with but one species, *T. humilis*, in S. Spain and Morocco. It is a tetraploid with $2n=28$, the same basic number therefore as the larger section in *Narcissus*. Morphologically however the *Tapeinanthus* chromosomes, with most centromeres subterminal, are closer to those of the $x=10$ (11) section of *Narcissus*. It forms a cytological bridge between the two sections and perhaps approximates to an ancestral condition from which these have subsequently diverged. There is no evidence so far that there has been a continuous series of basic numbers such as Neves (1939) reports for *Leucojum*. However the third genus in the tribe *Narcisseae*, *Cryptostephanus*, in which Gouws (1949) reports $2n=24$ for the S. Rhodesian *C. vansonii*, suggests the additional basic number for the tribe of $x=12$.

4. THE GARDEN NARCISSI

Narcissi have been cultivated since classical times, both as ornamental and medicinal plants, but the modern era in their horticultural development did not begin until the middle of last century, following the demonstration by Herbert (1843, 1847) of the possibilities of hybridisation. Fig. 4 shows the origin of the present-day groups of garden narcissi.

(i) *The Trumpet Group*

These are derived from members of the *pseudonarcissus* complex only. The first notable advance came in the 1860s, when triploid clones appeared independently among the seedlings of three English breeders, Backhouse, Leeds and Horsefield. These however did not come into general cultivation until 1875, when the collections of seedlings raised by these hybridisers were bought and classified by Barr (Barr, 1933). Information about the precise origin of these first triploids is lacking, since none of these breeders left records of their crosses. However, a note by Backhouse (1865) throws a little light on the question. He writes:

> "... *N. bicolor* seeds badly and is deficient in pollen, but from crosses of other daffodils with it, I have raised some of the largest and finest in the class. These also seed badly..."

This reference to the sterility of *bicolor* (a variety of *pseudonarcissus*) suggests that he had a triploid clone of this variety although the Fernandes (1946) report it as a tetraploid. The result of crossing this

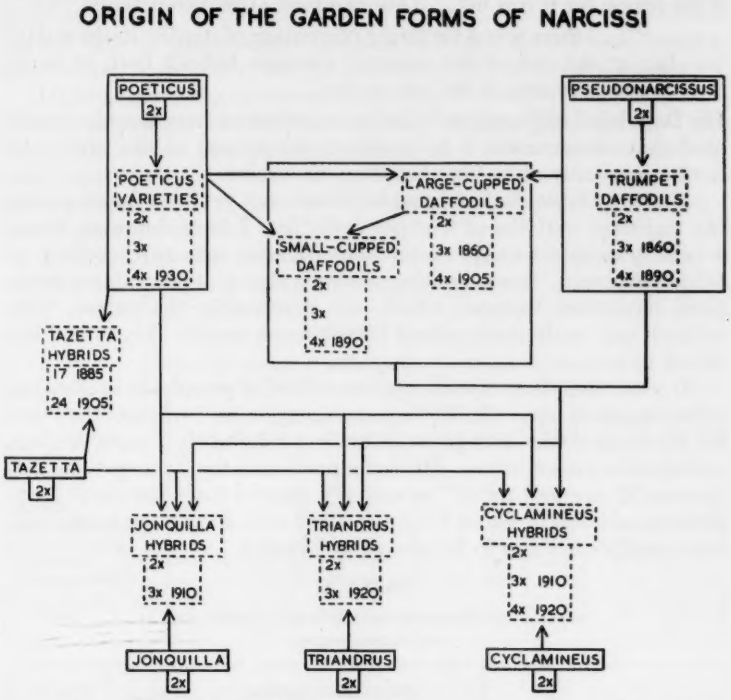

FIG. 4.—Diagram showing the origins of the main groups of garden narcissi.

triploid with a diploid variety of *pseudonarcissus* was to add the effect of hybrid vigour to that of polyploidy. The sister varieties " Emperor " and " Empress ", raised by Backhouse, were as we have seen triploid and were a great advance on any daffodil cultivated up to that time.

The triploids were the beginning of the new era in daffodil breeding. The tetraploid clones arose from them in the 1890s. The well known yellow Trumpet " King Alfred " was one of the first and attracted much attention when first shown. Again, its origin is uncertain. It may be " Empress " crossed with *N. pseudonarcissus hispanicus* (Williams, 1929), both triploids. I have found tetraploids in a family raised from " Emperor " self-fertilised; this origin is therefore quite plausible. The Dutch variety " Van Waveren's Giant " (1900), introduced only one year later than " King Alfred ", was found by de Mol (1923) to be a tetraploid also. The step from triploidy to tetraploidy evidently occurred independently at about the same time in the two main centres of daffodil breeding.

The tetraploid trumpet daffodils were of course more fertile than the triploids, which were all that had been available for breeding up till 1900. The comparative sterility of the latter was well known, even if the reason for it was not. Thus Williams (1929) comments:

> " . . . there was a far larger percentage of sterility in those days (i.e. at the end of last century) amongst hybrids both in males and females than is the case to-day."

His Darwinian explanation " that by selection we have largely eliminated that characteristic " is, however, the reverse of the truth: the fertiles inevitably arose from breeding the steriles.

Higher polyploids than tetraploids have not yet been found among the registered varieties of trumpet daffodils. I have, however, found a near hexaploid with 41 chromosomes among self-sown seedlings at Wisley (fig. 3*b*). It was growing among a clump of an ordinary tetraploid bicoloured trumpet, which was presumably the parent, from which it was easily distinguished by the much smaller flowers and less robust nature.

It seems therefore, that the optimum level of polyploidy in *Narcissus* is the tetraploid one. De Mol's query (1923) as to " whether the number of chromosomes may go on increasing indefinitely " must be then, answered in the negative. His assumption that the " conspicuous differences in size and form " in varieties derived from the early tetraploids would also be found to be associated with a variation in chromosome number has also so far proved unfounded.

TABLE 3

Comparison of chromosome numbers in the tetraploid varieties and in their progeny

	Chromosome number								Total	% with $2n=28$
	24	25	26	27	28	29	30	31		
Registered varieties										
I Species—Trumpets	—	—	1	2	37	1	1	1	**43**	*86·0*
II Hybrids—Large- and Small-cupped	—	—	—	4	99	1	—	—	**104**	*95·2*
Total	—	—	1	6	136	2	1	1	**147**	*92·5*
Seedlings										
1. Trumpet selfed	1	—	—	18	38	18	7	1	**83**	*43·5*
2. " "	—	—	—	4	10	1	1	—	**16**	*62·5*
3. Large-cupped open pollinated	—	—	—	4	6	7	—	—	**17**	*35·3*
Total	1	—	—	26	54	26	8	1	**116**	*46·5*

Table 3 shows the range in chromosome number found in the tetraploid trumpets, and also in the large- and small-cupped narcissi considered below. For comparison, the chromosome numbers found in families of seedlings from three different tetraploids are also included

in the table. A much higher percentage of the registered varieties have the balanced number of 28 than is the case in the seedlings. The horticulturally desirable forms have thus been selected for the balanced tetraploid number. It is unnecessary to invoke a hypothetical pentaploid to account for the origin of " Madame de Graaff ", a trumpet variety with 31 chromosomes (Fernandes, 1946), since I have found a plant with this number in the selfed progeny from a tetraploid (table 3).

Unfortunately the chromosomes cannot be divided into clearly differentiated classes, as they can in the hyacinths (Darlington, *et al.*, 1951). Doubtless the apparently balanced number of 28 conceals in some cases variation in the proportions of the different chromosome types present, such as these workers have demonstrated in the polyploid hyacinth varieties.

Table 4 gives the chromosome numbers found in pollen grains at the first mitosis in six tetraploids (2 trumpets and 4 large-cupped).

TABLE 4

Chromosome numbers in pollen grains of tetraploid varieties

Variety	Number of chromosomes								Total	Mean	% with $n=14$	Calculated loss
	10	11	12	13	14	15	16	17				
Beersheba .	—	—	—	16	**78**	4	2	—	**100**	13·9	*78·0*	
Bodilly . .	2	1	25	79	**133**	41	7	1	**289**	13·7	*40·0*	
Mrs E. H. Krelage	—	—	1	24	**138**	33	4	—	**200**	14·0	*69·0*	
Rustom Pasha .	—	5	21	**98**	65	10	1	—	**200**	13·3	*32·5*	
Warlock . .	—	—	18	95	**104**	29	4	—	**250**	13·6	*41·6*	
Whiteley Gem* .	—	—	6	37	**108**	37	7	—	**195**	14·0	*55·4*	
Total . .	**2**	**6**	**71**	**349**	**626**	**154**	**25**	**1**	**1234**	13·7	*50·7*	**3·7%**

* excluding the unreduced pollen grains

The proportion of grains with 14 chromosomes varies rather widely but is of the same order as that of seedlings with 28 chromosomes. If the female gametes have the same variation in chromosome number as the male, then comparison of the figures for pollen grains and for seedlings shows that there has been some selection against the more unbalanced gametes at fertilisation. But selection at this stage is evidently not as stringent as that practised by the breeder.

(ii) *The Poet Group*

The only other group to be derived solely from one major species is the much smaller one from varieties of *N. poeticus* intercrossed. Engleheart was the first to pay any attention to this group. His early hybrids, such as " Homer " and " Horace " were diploids like their parents, the varieties *ornatus* and *poetarum*. " Sarchedon ", a later diploid, is illustrated in fig. 6*b*. I found two of the later hybrids (1927 and 1939) to be tetraploids; polyploidy has thus been a factor in the development of this group as well. So far the only triploid known is

the variety *recurvus*, mentioned under the species. The recent tetraploids may therefore have arisen from the earlier diploid hybrids by chromosome doubling, as found by de Mol (1937) in one of the *pseudonarcissus-poeticus* hybrids, rather than by way of triploids, which seems to have been the more usual method in *Narcissus*.

(iii) *Trumpet-Poet Derivatives*

All the other horticultural groups of narcissi have arisen following crosses between major species or species complexes (fig. 4). *N. pseudonarcissus* and *N. poeticus* are the species responsible for the large groups of the large- and small-cupped narcissi, formerly known as the Incomparabilis and Barrii divisions respectively. The products of natural crosses had been cultivated for a long time with no idea that they were indeed of hybrid origin, until Herbert published his views. The first deliberate crosses with the aim of producing new garden varieties were made by Backhouse and Leeds (1850-1860), and introduced into commerce by Barr 20 years later. Again, imperfect records only were left and Engleheart (1890, 1894), dissatisfied with this uncertainty, decided to try to recreate these early garden hybrids by means of controlled crosses, in the same way that Herbert had done for the hybrid species half a century earlier. This he accomplished, confirming and considerably extending Herbert's results.

The large-cupped narcissi are primarily *pseudonarcissus* × *poeticus*. They are intermediate between the ancestral species in such characters as the relative length of the corona, the length of the perianth tube and the way in which the stamens are inserted, while the distinctive red pigment found in most *poeticus* varieties as a narrow rim at the edge of the very shallow corona, has been the source of all the red and orange shades in the hybrids. As Anderson and Hornback (1946) have shown, this same red pigment from *poeticus* is one of the factors responsible for the development of the pink colouring found in some recent hybrids.

The small-cupped varieties arose in the first place from backcrossing the large-cupped forms to *poeticus*, which they resemble in the length of the perianth tube and the obviously biseriate stamens (Engleheart, 1894). The small- and large-cupped classes may originally have been distinct, but after 70 years of hybridisation with each other and with the parental classes, there is now a continuous range between the two extremes.

Cytological data for the early representatives of these groups are meagre, but the outline is undoubtedly the same as for the Trumpet daffodils, with the early development of triploid clones, such as " Sir Watkin ", followed in the 1890s by tetraploid ones. Thus the tetraploid "Albatross " arose from the triploid " Empress " crossed with *N. poeticus*, presumably diploid (Bowles, 1934), while de Mol (1937) reports one tetraploid which arose as a somatic mutation of a diploid. Again tetraploidy is the highest level of polyploidy found. Table 3

shows that an even higher proportion of the large- and small-cupped narcissi than of the trumpet daffodils have the balanced chromosome number of 28. Conformity to the tetraploid number is more rigorously enforced in varieties of hybrid origin than in those derived from *pseudonarcissus* only, as would be expected. It seems as though the plant cannot break two rules at once.

(iv) *Other Hybrid Derivatives*

Four other species have taken part in the development of garden narcissi, though to a lesser extent than the two considered above (fig. 4).

(*a*) *N. cyclamineus*

Its hybrids are easily recognised, since the reflexing perianth segments of *cyclamineus* are usually present. It has been crossed with both diploids and tetraploids from the Trumpet and Large cupped groups, giving rise to diploid and triploid hybrids. " Minicycla " is an example of the former; it is from a cross with *pseudonarcissus* var. *asturiensis* (2*x*). " February Gold " and " Bartley " are examples of the latter, both of which have a yellow trumpet daffodil (4*x*) as the other parent.

I have also found one tetraploid, " Orange Glory ", which is Dutch in origin. It is reported to be *cyclamineus* crossed with a yellow trumpet; possibly a diploid gamete of *cyclamineus* was responsible for the origin of this tetraploid which would have the formula PsPsCC and be fertile. Little further breeding has yet been done from these primary hybrids, although even the triploids are moderately fertile. Col. Stern has raised progenies from " Bartley ". The seedling obtained from open pollination had 19 chromosomes, while two from a cross with the tetraploid large-cupped " Fortune " both had 26.

N. cyclamineus has also been crossed with *poeticus* or its varieties. One such hybrid is the triploid " Beryl " already mentioned in the section on B chromosomes. Since it is a fairly old variety (1907), it is not likely that the *poeticus* parent was a tetraploid, so that one of the parents must have contributed a diploid gamete.

There is also a hybrid recorded from a cross with *tazetta* (var. " Soleil d'Or "), named " Cyclataz ", which has 17 chromosomes. A diploid clone of " Soleil d'Or " must therefore have been the parent; I have found both diploid and triploid clones bearing this name.

(*b*) *N. jonquilla*

Besides the ancient hybrids of *jonquilla* with *pseudonarcissus* and *poeticus*, known as *N. odorus* and *N. gracilis* (fig. 2), there is a group of recent hybrids, derived from crosses with the new polyploid garden clones (fig. 4). They are characterised by dark green, narrow leaves, two- or three-flowered scapes and highly fragrant flowers. Most are triploids, the other parent having been a tetraploid, for example " Trevithian ". I have however found one tetraploid, " Yellow Prize ", again of Dutch origin.

The variety " Golden Perfection " has 31 chromosomes (fig. 5*b*). *Tazetta* was evidently one of the ancestors from the form of the chromosomes. The number 31 could have been achieved in either of two ways. An unreduced gamete in one of the triploid *poeticus-tazetta* ($2n=24$) hybrids, described below may have been fertilised by a normal *jonquilla* gamete ($n=7$), giving a hybrid with the constitution JoPoPoTz.

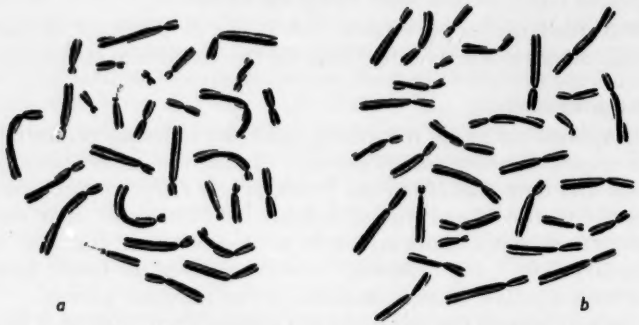

FIG. 5.—Root tip mitoses in two *tazetta* hybrids. (*a*) " Silver Chimes ", *triandrus* × *tazetta*, with 29 chromosomes (22+7). (*b*) " Golden Perfection ", a *jonquilla* cross with 31 chromosomes (17+14, or 24+7). × 1,750.

Alternatively, the parents could be a diploid poetaz hybrid ($2n=17$) and *N. jonquilla*, in both of which unreduced gametes were functional, producing the hybrid JoJoPoTz. The two cannot be distinguished cytologically, since the *jonquilla* and *poeticus* sets are alike in form.

Although a very distinct and desirable group of garden narcissi, no further breeding from these primary, mainly triploid, jonquil hybrids has yet been achieved, because of sterility (Wilson, 1948).

(*c*) *N. triandrus*

The two- or three-flowered scapes and the drooping habit of the flowers with slightly reflexed perianth segments are characters of *N. triandrus* usually found in its hybrids. There are small- and large-cupped varieties, depending on the group to which the second parent belongs. " Hawera " (*triandrus albus* × *jonquilla*) has the anticipated diploid number and the rest, except the one considered below, are triploids and evidently crosses with tetraploid varieties from the other groups. " Silver Chimes " has 29 chromosomes (fig. 5*a*) and has tazetta as the other parent. This number may have been arrived at by the combination of 7 chromosomes from the *triandrus* parent and 22 from the *tazetta*, through a diploid gamete. Since the hybrid is white, the *tazetta* parent presumably was one of the series Albae (Baker, 1888), of which all examined have 22 chromosomes (Fernandes, 1937*b*). As in the case of the *jonquilla* hybrids, sterility has proved an effective barrier to further breeding in this group.

(*d*) *N. tazetta*

The largest group of hybrids is derived from crosses with *poeticus* (fig. 4), corresponding with the natural hybrid *N. biflorus*. A good many of these " poetaz " varieties were raised at the beginning of this century in Holland and subsequently in this country as well. Cytologically the hybrids fall into two groups, with 17 and 24 chromosomes respectively. Examples are " Geranium " (fig. 6*c*) and " Glorious "

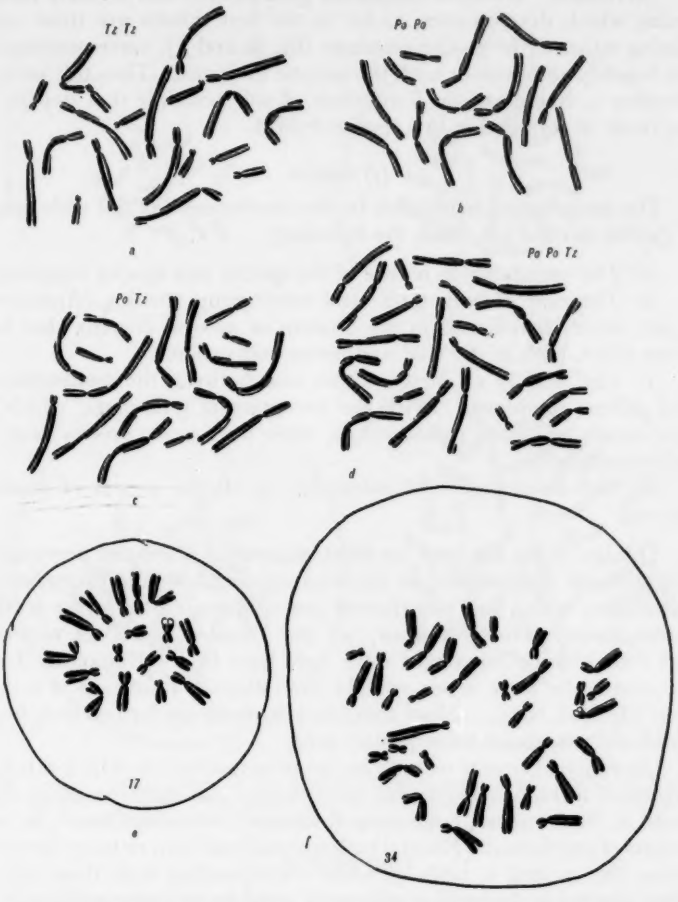

FIG. 6.—*N. poeticus*, *N. tazetta* and their hybrids. (*a*) the diploid *tazetta* " Soleil d'Or " with 20 chromosomes. (*b*) the diploid *poeticus* variety, " Sarchedon ", $2n=14$. (*c*) the diploid hybrid " Geranium " with 17 chromosomes (10+7). (*d*) the triploid hybrid " Glorious " with 24 chromosomes (10+14). (*e*) and (*f*) first pollen mitosis in " Geranium ": (*e*) diploid grain with 17 chromosomes, (*f*) a tetraploid one with 34. (*a*) - (*d*) × 1,400; (*e*) and (*f*) × 1,050.

(fig. 6*d*). The first class arose from crossing diploid varieties of both species, while in the second either a tetraploid *poeticus* variety was used or unreduced gametes from a diploid. Like the natural hybrids between members of the 7 and 10 series these garden hybrids are extremely sterile (Hannibal, 1945) and I have found no reports of their use in subsequent hybridisation, although, as noted above, there is cytological evidence that one of the 24 group may have taken part in the origin of the *jonquilla* hybrid " Golden Perfection ".

" Geranium " is almost completely pollen-sterile and the only pollen grains which develop even as far as the first mitosis are those containing either 17 or 34 chromosomes (fig. 6*e* and *f*), corresponding to the breakdown of one or both the meiotic divisions. Thus this variety provides a diagrammatic illustration of the principle that sterility is the result of segregation in a species hybrid.

(v) *General*

The main factors responsible for the development of this wide range of garden narcissi are, then, the following:

(*a*) The variability in nature of the species and species complexes.

(*b*) The ease of both inter- and intra-group crossing (Anderson, 1948), which is reflected in the amount of natural crossing that has taken place, both in the wild and under cultivation.

(*c*) The fertility of crosses within and between the *pseudonarcissus* and *poeticus* complexes. With the exception of *cyclamineus*, which is very closely related to *pseudonarcissus*, other inter-group crosses have so far proved sterile.

(*d*) The development of polyploidy in all the groups of garden narcissi.

This last factor has been the most important. As noted previously, polyploidy is insignificant in the wild species, with the exception of *bulbocodium*, which has contributed practically nothing so far to the garden forms. However nearly all the Trumpet and Poet varieties and their hybrids introduced since 1900 have been tetraploids. In a few cases these have arisen straight from diploids by means of a bud sport (de Mol, 1937). More commonly triploids are formed first, from which the tetraploids subsequently arise.

Unreduced gametes seem to be the principal way in which this has happened in both triploids and tetraploids. An extensive series was found in the tetraploid large-cupped variety " Whiteley Gem ", in two anthers of one flower. Normal pollen grains had from 12 to 17 chromosomes (fig. 7*a* and *b*, table 4) while corresponding with these was a series of giant pollen grains with even numbers of chromosomes from 24 to 32 (fig. 7*c* and *d*). Again, failure of the second division must have occurred. In addition to these classes of dividing pollen grains, there was a further group of larger ones still, which had already completed their divisions and which were probably formed by failure of

both meiotic divisions. They would be octoploid. In the *poeticus-tazetta* hybrids noted above, it is of course only the unreduced pollen grains which survive even till the first mitosis.

It is only the unreduced gametes produced by diploids, leading to the formation of triploids, and in these in turn producing the tetraploids, which have been functional so far. Those produced by the tetraploids themselves and by the poetaz hybrids have led to no further developments, with the exception of the hexaploid trumpet seedling from Wisley.

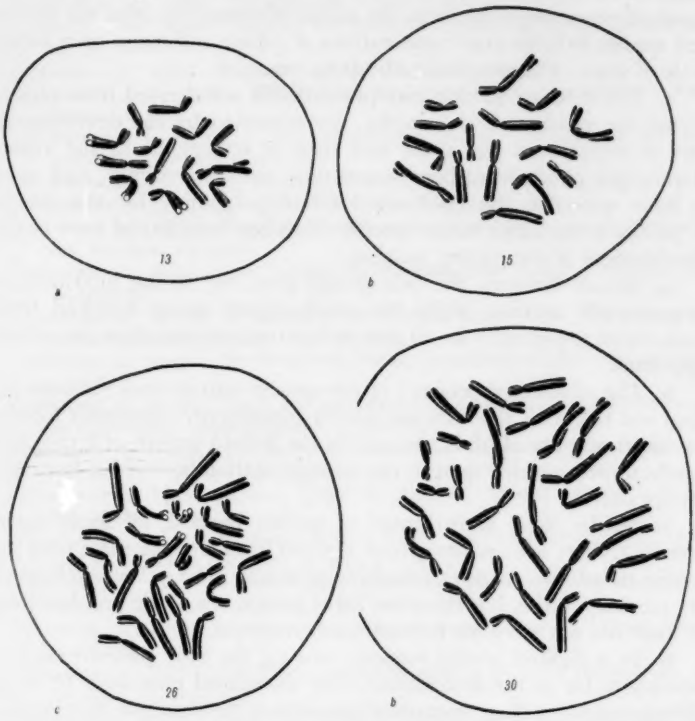

FIG. 7.—First pollen mitosis in the tetraploid large-cupped variety "Whiteley Gem". (*a*) and (*b*) haploid grains with 13 and 15 chromosomes respectively, (*c*) and (*d*) the corresponding unreduced grains with 26 and 30. × 1,050.

5. SUMMARY

1. There are several types of species in *Narcissus* which can be distinguished cytologically:

(*a*) Sexually Reproducing: the basic chromosome numbers, 10 and 11, of the *tazetta* group set them apart from the rest of the genus with 7.

(*b*) Clonal Hybrids: all sterile.

(*c*) Fertile Hybrids: the partially fertile diploid *bernardi*, from *pseudonarcissus* × *poeticus*, and the fully fertile hexaploid *dubius* ($2n=50$), from chromosome doubling in a *tazetta-juncifolius* hybrid.

2. Supernumerary or *B* chromosomes float in the populations of several sexual species and also exist in clones of some hybrid species and garden varieties. They may or may not be heterochromatic.

3. Polyploids are very few in *Narcissus* species but have been the main factor in the evolution of the garden varieties. During the last hundred years they have been the means of extracting from the species and species-hybrids new combinations of colour and form, on a larger scale of size. This process is still going on.

4. The modern garden trumpet daffodils are derived from crosses within the *pseudonarcissus* complex, accompanied by the development first of triploids in the 1860s and then of tetraploids in the 1890s. Higher polyploids have been found only among seedlings, and seem to have exceeded the optimum level of polyploidy for the group. *N. poeticus* is the other major species which has contributed most to the development of the garden narcissi.

5. Crosses between the two groups gave rise to the intermediate large-cupped narcissi, while the small-cupped group followed from backcrosses to *poeticus*. In all groups first triploids and then tetraploids appeared.

6. The unselected progeny of tetraploids contain more aneuploids than are found in the varieties finally introduced. Selection for the balanced number of 28 is stronger in the hybrid groups of large- and small-cupped narcissi than in the trumpet daffodils, derived from one species only.

7. Besides these main groups of garden narcissi, all freely interbreeding, there are smaller groups derived from crossing these with the species *cyclamineus*, *jonquilla*, *triandrus* and *tazetta*. The *cyclamineus* hybrids are partially fertile, but the other three groups are sterile and breeding in these has not yet gone beyond the first crosses.

8. In a diploid poetaz variety, $2n=17$, the only pollen grains to develop as far as the first mitosis were unreduced ones with 17 or 34 chromosomes—a diagrammatic illustration of the principle that sterility is the result of segregation in a species hybrid.

9. The newer groups of garden Narcissi may be expected to follow the course already described for the older groups, with increasingly diversified results.

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CHROMOSOME MOSAICS IN EXPERIMENTAL AMPHIPLOIDS IN THE *TRITICINAE*

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1. INTRODUCTION

DURING the course of an investigation on a series of experimental amphiploids, it was found that different chromosome numbers can occur in the same anther, with up to 27 chromosome numbers in the same plant. Cells with the different chromosome numbers were able to undergo meiosis, and in some cases form functional gametes. These gametes can then produce various chromosome number segregates from the same amphiploid parent. It was therefore decided to study the occurrence and distribution of chromosome variation in different plants and chromosome variation in the same plant. The present study describes these variations in a range of amphiploids involving *Triticum*, *Aegilops*, and *Agropyron*, three genera belonging to the subtribe *Triticinae*.

The anthers with different chromosome numbers contain at meiosis a mixture of cells with complete amphiploid and various reduced aneuploid chromosome complements. After the terminology of Fankhauser (1945) these tissues with different numbers can thus be described as " complex chromosome mosaics ".

2. MATERIAL AND TECHNIQUES

The following species have been used in the production of those amphiploids and amphiploid hybrids which have been studied.

$2n=14$	$2n=28$
Aeg. bicornis (Forsk.) Jaub and Sp.	*Aeg. cylindrica* Host.
Aeg. caudata L.	*Aeg. ovata* L.
Aeg. squarrosa L.	*T. timopheevi* Zhuk.
Aeg. umbellulata Zhuk.	*T. dicoccoides* Körn.

T. boeticum (Boiss.) Schiem.
(= *T. aegilopoides* (Link.) Bal.)

2n = 42

Agr. intermedium (Host.) Beauv.
T. aestivum L.
(= *T. vulgare* Vill.)
T. dicoccum Schübl.
T. durum Desf.
T. turgidum L.
T. polonicum L.
T. turanicum Jacub.
(= *T. orientale* Perc.)
T. carthlicum Nevski
(= *T. persicum* Vav.)

In the new amphiploids, *T. dicoccoides* from three different sources will be referred to as *T. dicoccoides* (a), (b) and (c).

Seeds of most of the new amphiploids were placed at my disposal by Dr G. D. H. Bell, and seeds of the later generation amphiploids were obtained from Dr E. R. Sears. All amphiploids were produced after colchicine treatment of F_1 hybrid plants.

For cytological observations, preliminary examinations of the anthers were made on temporary aceto-carmine smears. Permanent preparations of anthers were made by means of Feulgen squashes (Sachs, 1952). Root tip examinations were also made on Feulgen squashes.

TABLE 1

The Frequency of Mosaics at M_1 of Meiosis in 8x Amphiploids

NEW AMPHIPLOIDS		2n	No. of plants examined	No. of cells examined	Cells with reduced chromosome nos. %
Aeg. ovata	× *T. timopheevi* (i)	54	1	162	0·6
T. timopheevi	× *T. durum*	55	1	132	*0·8*
T. dicoccum	× *T. timopheevi* (i)	57	1	613	*1·1*
Aeg. cylindrica	× *T. carthlicum*	56	1	341	*1·2*
T. dicoccum	× *T. timopheevi* (ii)	55	1	647	*1·2*
,,	× *Aeg. ovata*	54	2	318	*1·9*
Aeg. cylindrica	× *T. durum*	56	3	764	*2·1*
,,	× *T. dicoccum*	{55	2	609	*2·1*
		{54	2	844	*2·4*
,,	× *T. carthlicum*	55	1	500	*2·4*
T. dicoccum	× *T. timopheevi* (iii)	56	2	979	*2·5*
T. timopheevi	× *T. turgidum*	56	1	503	*2·6*
Aeg. ovata	× *T. dicoccum*	55	1	782	*2·7*
T. dicoccoides (b)	× *T. timopheevi*	{55	1	341	*2·9*
		{ 56	3	1124	*3·4*
T. dicoccoides (a)	× ,,	56	3	1121	*3·8*
T. dicoccum	× *Aeg. ovata*	55	1	323	*4·0*
T. timopheevi	× *T. durum*	56	2	312	*4·8*
T. dicoccoides (a)	× *Aeg. ovata*	56	4	1179	*6·9*
T. diccocoides (c)	× ,, (i)	55	1	538	*7·3*
Aeg. ovata	× *T. dicoccum*	56	3	981	*8·9*
,,	× *T. timopheevi* (ii)	56	3	609	*14·1*
T. dicoccoides (c)	× *Aeg. ovata* (ii)	56	3	353	*14·5*

3. CHROMOSOME MOSAICS IN NEW AMPHIPLOIDS

With the exception of the 10*x* *T. dicoccum* × *Agr. intermedium*, all cytological observations on new amphiploids were made on first generation amphiploid plants. The amphiploid *T. dicoccum* × *Aeg. caudata* was grown in 1949 from the seed obtained after colchicine treatment of the F_1 hybrids in 1948. All other first generation amphiploids were grown in the same glasshouse in 1950 from the seed obtained after colchicine treatment of the F_1 hybrids in 1949. The 10*x* amphiploid

T. dicoccum × *Agr. intermedium* was studied in 1950 on a chromosome doubled part of the actual colchicine treated F_1 hybrid plants.

Chromosome counts at meiosis were made on anthers at the first metaphase (M_1) and the figures in the tables were obtained from Feulgen squashes. In the mosaic plants different anthers from the same plant gave significantly different frequencies of cells with aberrant chromosome numbers. Thus an analysis of variance test on different anthers of the same plant, in several amphiploids, gave significant values for heterogeneity. This variation was also found in different plants of the same amphiploid. There was thus a variation in the percentage of cells with aberrant chromosome numbers, both in different anthers of the same plant, and in different plants of the same amphiploid. This variation is probably due to the environment. The percentages of reduced cells in plants with chromosome mosaics must therefore be considered relative rather than absolute.

In plants with mosaics the majority of cells still contain one chromosome number, the other cells at meiosis containing a lower chromosome number. This majority number is thus referred to as the chromosome number of the plant. The observed frequencies of mosaics at M_1 of meiosis is shown in table 1 for 54-57 chromosome plants, and in table 2 for 41-42 chromosome plants. 37 different reduced chromosome numbers, ranging from 9 to 48, have been found in the amphiploids in table 1, with up to 29 different numbers in the same amphiploid and 27 different numbers in a single plant of *T. dicoccoides* (*a*) × *Aeg. ovata*. 23 different reduced chromosome numbers, ranging from 10 to 40, have been found in the new amphiploids in table 2, with up to 15 different numbers in the same amphiploid and 10 different numbers in a single plant of *Aeg. ovata* × *T. boeticum*.

Chromosome mosaics at meiosis were however *not* found in 5 new amphiploids which were examined. Thus there were no mosaics in the following:

924 cells of the 42 chr. *T. dicoccum* × *Aeg. caudata.*
333 cells of the 43 chr.
426 cells of the 42 chr. } *Aeg. caudata* × *T. dicoccoides* (b).
703 cells of the 41 chr.
335 cells of the 39 chr. *T. dicoccoides* (b) × *Aeg. caudata.*
211 cells of the 42 chr.
142 cells of the 41 chr. } *Aeg. cylindrica* × *T. boeticum.*
372 cells of the 70 chr. *T. dicoccum* × *Agr. intermedium.*

A comparison of the amphiploids shows that the presence of mosaics is dependent upon the particular parent species used in the production of the amphiploid. Thus e.g., the *T. boeticum* amphiploids gave mosaics if they included *Aeg. ovata* but no mosaics if they included *Aeg. cylindrica*.

The distribution of cells with different reduced chromosome numbers is shown in fig. 1. Data for later generation amphiploids and amphiploid hybrids have been included in the diagram for the 6*x* amphiploids. The diagram of the totals of reduced cells shows certain peak frequencies, such as the 42 and 35 chromosome peaks in the 8*x*

TABLE 2

The Frequency of Mosaics at M_1 of Meiosis in 6x Amphiploids

NEW AMPHIPLOIDS	2n	No. of plants examined	No. of cells examined	Cells with reduced chromosome nos. %
Aeg. caudata × *T. dicoccum*	{42	3	2639	0·1
	{41	2	1746	0·1
" " × *T. turgidum*	42	4	2520	0·1
T. dicoccoides (c) × *T. boeticum*	41	1	739	0·3
Aeg. caudata × *T. durum*	42	3	1106	0·7
T. dicoccoides (c) × *T. boeticum*	42	3	1238	1·5
Aeg. caudata × *T. timopheevi*	42	4	689	3·1
Aeg. ovata × *T. boeticum*	42	4	816	3·2
LATER GENERATION AMPHIPLOIDS				
T. dicoccoides × *Aeg. umbellulata*	42	1	241	0·8
T. timopheevi × *Aeg. bicornis*	42	5	613	5·5
AMPHIPLOID HYBRIDS				
T. aestivum var. April Bearded × (*T. dicoccoides* × *Aeg. umbellulata*)	42	1	84	2·4
(*T. dicoccoides* × *Aeg. umbellulata*) × (*T. dicoccoides* × *Aeg. caudata*)	42	1	91	4·4

amphiploids. These peaks in the 8*x* diagram are, however, derived from only 2 out of 13 amphiploids with mosaics. There is thus a peak of 42 chr. cells in the 56 chr. *Aeg. ovata* × *T. timopheevi*, and a peak of

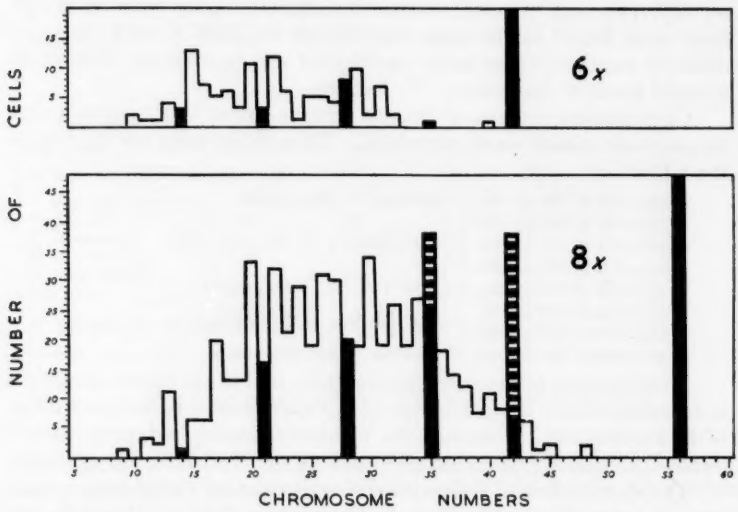

FIG. 1.—Diagrams of the frequencies of cells with different chromosome numbers.
Upper diagram = 6*x* amphiploids.
Lower diagram = 8*x* amphiploids.
Cross-hatching in 8*x* diagram = the 35 and 42 chr. cells from two particular plants with peak frequencies.

35 chr. cells in the 56 chr. *T. timopheevi* × *T. durum*. In addition to peaks occurring in only two out of the 13 amphiploids, the 42 peak in *Aeg. ovata* × *T. timopheevi* was found in only one of the three plants of this amphiploid and the 35 peak in *T. timopheevi* × *T. durum* in only one of two plants. The peaks in these two plants may have been produced by earlier abnormalities than those occurring in the other plants.

Altogether there was a peak in the frequency of cells with a particular reduced chromosome number in only two out of forty-three plants with mosaics shown in table 1. Thus 31 cells with 42 chrs. have been found in one plant, and 12 cells with 35 chrs. have been found in the other plant. These cells have been marked by cross-hatching in the 8*x* diagram (fig. 1). If these 42 and 35 chr. cells, derived from the two plants, are not included in the 8*x* totals, the diagram loses its peaks at 35 and 42. There is thus, in general, no regularity in the formation of frequency peaks either in the 8*x* or in the 6*x* amphiploids.

4. CHROMOSOME MOSAICS IN LATER GENERATION AMPHIPLOIDS AND THEIR HYBRIDS

Chromosome mosaics at meiosis have also been found in later generation amphiploids. The amphiploids *T. dicoccoides* × *Aeg. umbellulata* and *T. timopheevi* × *Aeg. bicornis* were produced by MacFadden and Sears (1947) in 1941 and 1942 respectively, and in addition to having been grown at Missouri, U.S.A., they have also been grown at Cambridge, England, for two further generations. It can be seen from table 2 that chromosome mosaics were found at meiosis in both these amphiploids.

In the amphiploids *T. timopheevi* × *Aeg. bicornis* mosaics at meiosis were found in 42 chromosome plants grown at Cambridge, England, in 1949 and 1950, and at Lund, Sweden, in 1951. With seeds which were also obtained from Dr E. R. Sears, Li and Tu (1947) found mosaics in this amphiploid in plants grown at Szechuan, China. Variation in chromosome number at meiosis has thus been observed in successive generations and under a range of environmental conditions. In addition to the two later generation amphiploids mentioned above, mosaics were also observed in a 42 chromosome plant of the later generation amphiploid *T. timopheevi* × *Aeg. squarrosa*.

The data in table 2 further shows that mosaics were found in a hybrid between the amphiploid *T. dicoccoides* × *Aeg. umbellulata* and a variety of *T. aestivum*.—No mosaics were found in hybrids between the amphiploid *T. dicoccum* × *Aeg. caudata* and four varieties of *T. aestivum*. The ability of the amphiploid *T. dicoccoides* × *Aeg. umbellulata* to produce a small percentage of cells with reduced chromosome numbers was thus also found in the hybrid with *T. aestivum*. No mosaics were found in the amphiploid *T. dicoccum* × *Aeg. caudata* and there were also no mosaics in the hybrids with *T. aestivum*. Cells with reduced chromosome numbers were also found in a hybrid between the amphiploid

T. dicoccoides × *Aeg. umbellulata* and the amphiploid *T. dicoccoides* × *Aeg. caudata*. As in the new amphiploids, there is no regular formation of peaks in the frequency of cells with a particular reduced chromosome number. In the amphiploid *T. timopheevi* × *Aeg. bicornis*, a slight peak in the frequency of 15 chromosome cells was found but only in one of the five plants examined.

TABLE 3

Amphiploid Chromosome Pairing and the Percentage of Cells with Reduced Chromosome Numbers

AMPHIPLOIDS	Configurations in cells with complete amphiploid chromosome no. I	II	III	IV	V/VI	Cells with reduced chromosome nos. %
2n=42						
Aeg. cylindrica × *T. boeticum*	1·52	20·24	—	—	—	—
Aeg caudata × *T. dicoccoides*(b)	0·40	20·80	—	—	—	—
T. dicoccum × *Aeg. caudata*	0·12	20·94	—	—	—	—
Aeg. caudata × *T. dicoccum*	0·12	20·94	—	—	—	*0·1*
,, × *T. turgidum*	0·38	20·66	0·10	—	—	*0·1*
,, × *T. durum*	0·68	20·60	0·04	—	—	*0·7*
T. dicoccoides (c) × *T. boeticum*	1·48	18·90	0·24	0·50	—	*1·5*
Aeg. caudata × *T. timopheevi*	1·50	20·16	0·06	—	—	*3·1*
Aeg. ovata × *T. boeticum*	0·44	20·78	—	—	—	*3·2*
2n=56						
Aeg. cylindrica × *T. carthlicum*	7·04	24·48	—	—	—	*1·2*
,, × *T. durum*	6·76	24·62	—	—	—	*2·1*
T. dicoccum × *T. timopheevi*	1·04	23·94	0·24	1·56	0·02	*2·5*
T. timopheevi × *T. turgidum*	0·92	24·06	0·18	1·58	0·02	*2·6*
T. dicoccoides (b) × *T. timopheevi*	1·48	23·28	0·36	1·72	—	*3·4*
T. dicoccoides (a) × ,, *	1·47	21·10	0·47	2·73	—	*3·8*
T. timopheevi × *T. durum*	1·70	23·18	0·38	1·70	—	*4·8*
T. dicoccoides (a) × *Aeg. ovata*	3·64	26·18	—	—	—	*6·9*
Aeg. ovata × *T. dicoccum*	4·20	25·90	—	—	—	*8·9*
,, × *T. timopheevi*	1·50	27·20	—	—	—	*14·1*
T. dicoccoides (c) × *Aeg. ovata*	0·60	27·70	—	—	—	*14·5*
2n=70						
T. dicoccum × *Agr. intermedium*	1·24	34·38	—	—	—	—

* 30 cells, in all others 50 cells analysed for chromosome pairing in each amphiploid.

5. CHROMOSOME MOSAICS AND AMPHIPLOID PAIRING

It has been shown in the previous sections that chromosome mosaics can be found at meiosis in the anthers of some amphiploids, and that different amphiploids show different percentages of cells with reduced chromosome numbers. An analysis of chromosome pairing at meiosis in cells with the complete amphiploid chromosome complement has shown that different amphiploids have different degrees of chromosome pairing. It is therefore of interest to determine if the presence of cells with reduced chromosome complements affects the chromosome pairing at meiosis in cells with the complete chromosome complement. Chromosome pairing in cells with the complete amphiploid chromosome complement has therefore been recorded in relation to the percentage of

cells with reduced chromosome numbers. This is shown in table 3.

A comparison of 21 different amphiploids shows that amphiploid chromosome pairing is not related to the presence or frequency of cells with reduced chromosome numbers. Chromosome pairing at meiosis in cells with the complete amphiploid chromosome complement is thus determined by the combination of parent species and varieties used in the production of the amphiploid.

6. PAIRING IN CELLS WITH A REDUCED COMPLEMENT

A high univalent frequency is characteristic of cells with a reduced chromosome complement. It is found independently of the presence of univalents in cells with the complete amphiploid chromosome complement. If, however, multivalents are formed in cells with the complete amphiploid complement, multivalents also occur in some cells with a reduced complement. Pairing has been studied in all the cells with reduced chromosome complements, and this has shown a wide variation in their constitution. Some representative data on pairing is given in table 4.

TABLE 4

Chromosome Pairing in Cells with Reduced Chromosome Numbers

SPECIES HYBRID 6x *T. dicoccoides* (c) × *T. boeticum*					GENUS HYBRID 8x *T. dicoccoides* (c) × *Aeg. ovata*			
Chr. no. of cell	Chromosome pairing			Frequency	Chr. no. of cell	Chromosome pairing		Frequency
	I	II	III			I	II	
10	10	—	—	1	11	11	—	1
11	9	1	—	1	13	11	1	1
13	7	3	—	1	17	15	1	3
15	11	2	—	2	„	13	2	2
16	10	3	—	2	20	14	3	3
17	11	3	—	1	„	10	5	2
20	8	6	—	3	21	13	4	1
„	7	5	1	1	23	13	5	1
21	7	7	—	1	28	14	7	1
22	12	5	—	1	„	12	8	2
„	7	6	1	1	„	8	10	2
23	5	9	—	1	35	15	10	2
31	9	11	—	1	42	14	12	2

The general absence of frequency peaks at particular chromosome numbers in the mosaics and their high univalent frequency indicates that the reduced numbers represent an almost random assortment of chromosomes. This could have been produced by pre-meiotic spindle abnormalities. Chromosome pairing at meiosis in undoubled F_1 hybrids and in complete amphiploid cells has shown a greater pairing affinity between chromosomes in the interspecific hybrids than in the intergeneric hybrids. A random assortment of chromosomes in the interspecific amphiploids should thus show a greater degree of pairing than in the intergeneric amphiploids. These different degrees of chromo-

some pairing are in fact shown in table 4. Thus in the same plant meiosis can take place in cells which differ widely in their chromosome constitution.

7. THE SURVIVAL OF ABERRANT GAMETES

The present data on the survival of aberrant gametes is based on the chromosome constitution of 112 first generation amphiploid plants. The complete amphiploid number is 42 for the plants in table 5, and 56 for those in table 6. The chromosome constitutions of these plants were determined at M_1 of meiosis. It can be seen from table 5 that out of a total of 36 plants, 16·7% had less than 42 chromosomes, and there was one plant with 43 chromosomes. The existence of sub-amphiploid numbers shows that, with a slight reduction in chromosome number, these amphiploids may still produce functional gametes. Comparable data for different amphiploids in the Triticinae have also been given by MacFadden and Sears (1947) and others. The survival of gametes with a reduced chromosome complement is further seen in table 6 where 66 plants gave 33·3% with a sub-amphiploid chromosome number.

TABLE 5

Chromosome Numbers of First Generation Plants whose Complete Amphiploid Chromosome Number would be 42

AMPHIPLOID		No. of plants examined	Chr. no. of 1st generation plants			
			39	41	42	43
Aeg. ovata	× *T. boeticum*	5	—	—	5	—
Aeg. caudata	× *T. timopheevi*	5	—	—	5	—
,,	× *T. durum*	4	—	—	4	—
,,	× *T. turgidum*	4	—	—	4	—
T. dicoccum	× *Aeg. caudata*	2	—	—	2	—
Aeg. caudata	× *T. dicoccum*	5	—	2	3	—
,,	× *T. dicoccoides* (b)	3	—	1	1	1
T. dicoccoides (b)	× *Aeg. caudata*	1	1	—	—	—
T. dicoccoides (c)	× *T. boeticum*	4	—	1	3	—
Aeg. cylindrica	× ,,	3	—	1	2	—
Total		36	1	5	29	1

Of particular interest, however, are the 28, 44, 48, and one of the 54 chromosome plants. The 28 plants derived from *T. timopheevi* × *T. turanicum* is comparable both in chromosome number and in chromosome pairing to its 28 chromosome F_1 hybrid. In this plant, haploid parthenogenesis (cf. Darlington, 1937) may have restored the original F_1 balance. In this 28-chromosome plant with the original F_1 balance and number, I find there are no longer any mosaics in the anthers.

The four plants in table 7 have also been derived from 28 chromosome F_1 plants, but they show a marked deviation in number and pairing both from the F_1 hybrid and the expected amphiploids. These

TABLE 6

Chromosome Numbers of First Generation Plants whose Complete Amphiploid Chromosome Number would be 56

AMPHIPLOID	No. of plants examined	Chr. no. of 1st generation plants						
		28	44	48	54	55	56	57
T. dicoccoides (a) × *Aeg. ovata*	5	—	—	—	—	—	5	—
Aeg. cylindrica × *T. durum*	3	—	—	—	—	—	3	—
T. dicoccoides (a) × *T. timpopheevi*	4	—	—	—	—	—	4	—
T. turgidum × ,,	3	—	—	—	—	—	3	—
T. timopheevi × *T. turgidum*	2	—	—	—	—	—	2	—
,, × *T. polonicum*	2	—	—	—	—	—	2	—
T. timopheevi × *T. durum*	3	—	—	—	—	1	2	—
T. dicoccum × *T. timopheevi*	6	—	—	—	—	1	4	1
T. dicoccoides (b) × ,,	4	—	—	—	—	1	3	—
Aeg. cylindrica × *T. carthlicum*	3	—	—	—	—	1	2	—
,, × *T. dicoccum*	4	—	—	—	2	2	—	—
T. dicoccoides (c) × *Aeg. ovata*	5	—	—	—	1	1	3	—
Aeg. ovata × *T. timopheevi*	4	—	—	—	1	—	3	—
,, × *T. dicoccum*	4	—	—	—	—	1	3	—
,, × *T. dicoccoides* (b)	1	—	1*	—	—	—	—	—
T. dicoccum × *Aeg. ovata*	4	—	—	1*	2	1	—	—
T. timopheevi × ,,	5	—	—	1*	—	2	2	—
T. durum × ,,	2	—	—	—	1*	—	1	—
T. timopheevi × *T. turanicum*	2	1	—	—	—	—	1	—
Total	66	1	1	2	7	11	43	1

*=data on chromosome pairing in these plants in given in table 7

plants are characterised by a high univalent frequency, and they must have been derived from the fusion of gametes with deviating chromosome complements. Nevertheless, mosaics at meiosis have been found in these 44, 48, and 54 chromosome plants. These mosaics in the anthers are similar to those which have been described for the amphiploid plants.

The origin and union of similar types of aberrant gametes would explain the occurrence of plants with an abnormal chromosome constitution such as those reported in *Triticum-Agropyron* hybrids (Love and Suneson, 1945).

TABLE 7

Chromosome Pairing in Aneuploid Plants derived from Reduced Cells in the Mosaics

8x PARENTS	Approx. no of I per cell in 8x parent	Aneuploid chr. no.	Chromosome pairing Aneuploids		
			I	II	III
Aeg ovata × *T. dicoccoides* (b)	**0·6**	44	**26·04**	8·98	—
T. dicoccum × *Aeg ovata*	**4·2**	48	**19·44**	14·28	—
T. timopheevi × *Aeg. ovata*	**1·5**	48	**25·62**	9·66	1·02
T. durum × *Aeg. ovata*	**2·8**	54	**16·44**	17·40	0·92

50 cells analysed in each aneuploid plant

In the present investigation the plants with an aberrant chromosome constitution (table 7) were sterile. This sterility resulted from a high univalent frequency. Owing to the almost random assortment of chromosomes in the cells at meiosis, the same sterility would be expected in many plants produced in a similar manner. In annual sexually reproducing plants, where perpetuation in nature is dependent upon sexual reproduction, many of these abnormal constitutions would be unable to survive on account of their sterility. In perennial plants, the abnormal constitution could survive in nature despite sterility. The survival of these plants with varying chromosome constitution could ensure the production of gametes with a further deviation from the original chromosome condition. Thus the four plants with an abnormal constitution were still able to produce mosaics at meiosis. In perennial plants, therefore, a similar mechanism could in time produce an extensive range of aneuploidy and a reduction of chromosome number in phylogeny. A very extensive aneuploid series has in fact been found in some plant genera (Stebbins, 1950, table VIII). The mechanism of chromosome variation described above could thus explain the origin and establishment of some of these aneuploid series which have been found to occur in nature.

8. DISCUSSION

(a) *The Origin of Chromosome Mosaics*

The present investigation has shown that cells with reduced chromosome numbers can be found at meiosis in certain amphiploids. These chromosome mosaics at meiosis were not found either in the parent species or in the F_1 hybrids from which the amphiploids were produced. They were also not found in all amphiploids. No mosaics were found in the 70 chromosome anthers, the highest chromosome number amphiploid which was examined. The existence of mosaics was therefore not dependent merely upon the magnitude of the chromosome number of the amphiploids.

It has further been shown that the capacity to form mosacis can be transmitted to successive generations. All amphiploids in the present investigation were produced by the colchicine treatment of F_1 hybrid plants. The mosaics at meiosis in the present study can however not be attributed to a permanent effect of colchicine such as that suggested by Vaarama (1949*a*) in *Ribes nigrum*. Cells with reduced chromosome numbers at meiosis have occasionally been observed in other *Triticum* hybrids which had not been treated with colchicine (Hollingshead, 1932; Love, 1938) and in a *Triticum-Secale* hybrid which was also produced without colchicine (Katterman, 1933). A more probable explanation, therefore, is that the mosaics depend on certain gene combinations.

Cells with reduced chromosome numbers have been found at the early stages of meiosis. The mosaics must therefore have been produced by irregularities at the pre-meiotic cell divisions. The different

chromosome numbers at meiosis could have been produced by spindle abnormalities, such as multipolar spindles, during these pre-meiotic cell divisions. That certain gene combinations can cause split spindles has been established both for mitosis and meiosis. Darlington and Thomas (1937) have shown that abnormal spindles at meiosis can occur in a hybrid derivative of *Festuca-Lolium*. Spindle abnormalities at meiosis can be determined by single gene differences (Smith, 1942). Certain gene combinations can produce multipolar spindles at mitosis (Vaarama, 1949*b*). The genetic control of spindle abnormalities at mitosis would explain the distribution and transmission of chromosome mosaics in amphiploids. As different amphiploids possess different gene combinations, this would explain the presence of mosaics in some and their absence in others. In addition, the genotype of an amphiploid differs from that in its F_1 hybrid and its parent species. The absence of mosaics in parent species and the F_1 hybrids and their presence in an amphiploid are thus a reflection of the differences in their genotypes. The occurrence of cells with reduced chromosome numbers at meiosis in one strain of autotetraploid rye (O'Mara, 1942), and the absence of these cells in another strain of autotetraploid rye (Müntzing, 1951), may also be due to the difference in their genic combinations.

The distribution at meiosis of cells with reduced chromosome numbers can give some indication of the time at which earlier disturbances have taken place. In amphiploids with mosaics, the cells with reduced numbers occurred at meiosis, either in small groups, or singly—even in the same anther. In addition, the reduced cells were not localised in any particular position in the anthers. It has been pointed out above that in the anthers with mosaics the majority of cells still contain the non-reduced number, which was constant for each plant in all its anthers. There was thus no case of a chromosome chimaera between different anthers of the same plant. Moreover there were no mosaics at mitosis in the root tips in plants with mosaics at meiosis. All these things indicate that the mitotic irregularities must have occurred late in the development of the plant. They could thus have been produced in the cell generations just preceding meiosis.

The cell divisions immediately preceding meiosis may in fact be different from the mitosis occurring at other stages in ontogeny. A precocity theory of meiosis has been suggested by Darlington (1937, 1939). According to this theory, the external changes outside the chromosomes are precocious at meiosis. Oksala (1944) has concluded that precocity in the dragonfly *Aeschna juncea*,, is gradually developed and can be observed in at least two cell divisions preceding meiosis. Mitosis in cells with this developing precocity is therefore different from mitosis in other cells. Mitotic abnormalities may thus occur during these pre-meiotic cell divisions although they do not occur at other stages of ontogeny. However not all gene combinations producing mitotic abnormalities only function at a late stage in ontogeny. Other

gene combinations, such as those in tetraploid *Ribes nigrum* (Vaarama, 1949*b*) produce mitotic abnormalities resulting in different chromosome numbers in the root tips. Similar mitotic abnormalities can therefore be produced by different gene combinations at different stages in ontogeny.

In the present study there has been no evidence of chromosome pairing and segregation in somatic tissues. Huskins (1949) has however suggested that cells with reduced chromosome numbers can be produced by somatic chromosome pairing and segregation. But his cited evidence in plants cannot be considered as conclusive. The recent evidence on "somatic reduction" in some *Gossypium* hybrids (Menzel and Brown, 1952) can also be explained if the hybrid genotypes produce multipolar spindles. I have attempted to confirm the induction of " somatic meiosis " and " chiasma formation " in somatic tissues by treatment with sodium ribose nucleate. Root tips of *Allium cepa* and *Triticum monococcum* were treated with sodium salts of both ribose and deoxyribose nucleic acid in various concentrations. These have shown no evidence for a " somatic meiosis ". It must therefore be concluded that somatic chromosome pairing and segregation in plants has not yet been cytologically demonstrated.

(*b*) *Aneuploid Variation within the Tissue*

The existence of aneuploid variation in the same tissue shows that cells with a considerable deviation from the full chromosome complement are still able to divide. In the amphiploids examined, cells containing a variety of different chromosome numbers were able to undergo meiosis in the same plant. The greatest difference observed was the existence of 9 and 56 chromosome cells at meiosis in the same anther. The division of these cells at meiosis was almost completely synchronised so that the cells with different chromosome numbers are undergoing meiosis at the same time.

The synchronisation of the different cells at meiosis can be understood if the initiation of the process of meiosis is connected with the diffusion of certain substances. Montalenti *et al.* (1950) have suggested that in the testis of *Asellus aquaticus*, ribose nucleic acid secreted from outside the germ cells is casually connected with the beginning of meiosis. In this connexion it is of interest that the secretory cells in *Asellus*, which surround the cells undergoing meiosis, are polyploid. In plants the tapetum, which surrounds the cells undergoing meiosis, also contains polyploid cells (Brown, 1949). In the present study it has been observed that the tapetal cells have only one nucleus before the beginning of meiosis, but that the tapetal cells are binucleate during meiosis. The polyploid tapetal cells may thus fulfil the same secretory function in the anthers which has been attributed to the polyploid secretory cells of the testis in *Asellus*.

The simultaneous initiation of meiosis in the cells with different chromosome numbers, connected with the diffusion of certain sub-

Microphotographs of Pollen mother cells at Metaphase 1.
Feulgen stained × 1100.

1. 6x *Aeg. ovata* × *T. boeticum*
14 chromosome cell in 42 chr. plant.
6 I 4 II

2. 6x *T. timopheev i* × *Aeg. bicornis*
22 chromosome cell in 42 chr. plant.
12 I 5 II

3. 8x *Aeg. ovata* × *T. dicoccum*
16 chromosome cell in 56 chr. plant.
12 I 2 II

4. 8x *Aeg. ovata* × *T. dicoccum*
40 chromosome cell in 56 chr. plant.
12 I 14 II

5. 6x *Aeg. caudata* × *T. turgidum*
42 chromosome cell in 42 chr. plant.
3 I 18 II 1 III

6. 8x *Aeg. ovata* × *T. timopheevi*
56 chromosome cell in 56 chr. plant.
4 I 26 II

stances, can thus account for the ability of the aneuploid cells to undergo meiosis. The diffusion of certain substances can also account for the ability of the aneuploid cells to undergo mitosis. Another cause of the survival of aneuploid cells in the amphiploids is that the derived polyploid origin of the plants concerned may have been connected with a greater ability to tolerate the loss of chromosomes. In addition, different plant species may show different degrees of chromosome differentiation (Darlington and Mather, 1944).

In animals also, different species may differ in the physiological differentiation of their chromosomes. In addition, animal tissues with no proper cell wall present considerable possibilities for the diffusion of substances between different cells. The diffusion of substances between the different cells could thus account for the high viability of aneuploid numbers which have been found in certain human tissues (Therman and Timonen, 1951).

The available data on aneuploid variation in the same tissue is, however, still very small. A further study of the development and extent of this chromosome variation, and of the possible substances involved in cell survival, would greatly contribute to an understanding of the processes of growth, development, and differentiation.

9. SUMMARY

1. Chromosome mosaics at meiosis arise in the anthers of 19 out of 24 new amphiploids in *Triticum*, *Aegilops*, and *Agropyron*.

2. The mosaics occurred in all the 8*x*, in some of the 6*x*, but not in a 10*x* amphiploid.

3. The anthers with mosaics contain at meiosis a mixture of cells with complete amphiploid and various reduced aneuploid numbers.

4. 37 reduced chromosome numbers, from 9 to 48, were found in the 8*x* amphiploids, and 24 reduced numbers, from 10 to 40, in the 6*x* amphiploids. 27 numbers at meiosis occurred in a single 8*x* plant, and 10 numbers at meiosis in a single 6*x* plant.

5. There was no relationship between the presence of mosaics and chromosome pairing in those cells which have retained a complete chromosome complement.

6. The reduced cells in mosaic plants represent at meiosis an almost random assortment of chromosomes. Mosaics probably arise by gene-controlled spindle abnormalities just before meiosis.

7. Plants bred true for mosaic formation. Moreover, reduced cells in mosaic plants can function as gametes. Their progeny can again produce mosaics. A range of chromosome numbers can thus be derived from the same amphiploid.

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POPULATION AND SELECTION STUDIES IN A *TRITICUM* CROSS

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1. INTRODUCTION

YIELD increases are achieved in breeding programmes in two main ways, as pointed out by Frankel (1950); by removing or reducing the effects of factors which limit yield, which he called resistance breeding, or by direct selection for increases in yield itself, called production breeding. The present paper deals with some problems encountered in the second of these aspects of breeding.

The inheritance of yield, as of many other characters, is controlled by polygenic systems. The essential feature of such systems is that they are made up of many genes which have effects small in comparison with environmental variations, the individual polygenes occurring in more or less closely linked groups (Mather, 1942). Because environmental variations contribute more to the phenotype than any single polygene, superior polygenic combinations are often not observable in single plants or their immediate progenies, and in this way they contrast with oligogenes.

No character can be placed in either class unconditionally; oligogenes have polygenic modifiers, and, in some crosses, a group of closely linked polygenes might act as an oligogene. If genes interact with other genes or the environment, and gene action accordingly is not arithmetic, genetic changes observable at one level of expression may be unobservable at another. For example, where the environment limits development, increases in yield potential may not be observable.

Since genetic differences between plants are masked by environmental influences, selection of high yielding plants in the early generations after a cross is unlikely to be accurate. The chance of obtaining genetically superior plants from any population by phenotypic selection depends on their frequency, their relative phenotypic advantage, and the extent to which this advantage is genetically determined and fixable. The aim of the breeder is to produce and use populations from which the chance of successful selection is highest.

When breeding for increased yields of self-fertilised crops, two alternative methods—the bulk or mass method, and the pedigree method—are commonly used. Both methods, and modifications of them, have been adequately described (Harrington, 1937; Akerman and MacKey, 1948). Briefly, with the pedigree method, single plant selection begins

in F2 and is continued until progenies are considered to be homozygous for observable characters. When this stage is reached, usually after two or more generations of single plant selection, progenies of selected plants are tested. With the bulk method, single plant selection begins when the majority of plants is considered to be already homozygous, and is often confined to the one generation.

Various changes occur in bulked populations from F2 onwards. Two forces with predictable effects are acting; segregation and natural selection. The precise genetic effects of segregation depend on the number of genes segregating, their linkage and dominance relationships, and the magnitude and direction of gene interaction. Whatever the genetic situation, the general effects of segregation can be predicted.

The proportion of homozygotes in the population increases from F2 onwards according to the well known formula $\left[\frac{2^r - 1}{2^r}\right]^n$, where r = the number of generations after F1 and n = the number of independently segregating genes. Linkage in effect reduces the number of segregating genes, and in either the coupling or repulsion phase increases the rate of attainment of homozygosis. The increase in the proportion of homozygotes, with the consequent elimination of heterozygotes, increases the phenotypic variance of the population. With arithmetic gene action, no dominance and no gene interaction, the variance when segregation has ceased is double that of F2 (Wright, 1921). With some other genetic systems the increase in variance is not so great, but it occurs with all systems.

The effect of segregation on the population mean depends on the dominance relationships of the yield genes. If dominance is absent, the population mean remains unchanged from generation to generation. If dominance occurs, the mean in each succeeding generation approaches more closely to the mean of the parents of the cross as heterozygotes are eliminated.

Out crossing reduces the effects of segregation, but at a rate likely to be met with in most self-fertilised plants its effect on population structure is negligible. Bateman (1951) assuming 3% of outcrossing in each generation, states that this could greatly increase the heterozygosis of late generation plants. His assumption that natural selection favours heterozygotes must be generally invalid for self-fertilised plants. Ignoring any such effect, if 20 genes are segregating the proportion of complete homozygotes in F8 is reduced from 85% to 48% by 3% of out-crossing. But 84% of the population will be homozygous for 19 or more genes, with most of the remaining 16% homozygous for 18 genes.

Natural selection in seed crops generally favours those genotypes producing the greatest number of seeds, so, if seed weight and seed number are not inversely correlated, it favours the higher yielding genotypes. It thus tends to increase the population mean and to reduce its genetic variance.

The effects of natural selection and segregation are revealed not only by the means and variances of F_2 and later generations but also by the parent-offspring correlations of F_2 and later generation plants. If dominance occurs, progeny of heterozygotes tend to have lower mean yields than their parents, while progeny of homozygotes have the same yield as their parents. Consequently parent-offspring correlations of early generation plants, many of which are heterozygous, tend to be lower than those of late generation plants, which are mainly homozygotes. If no dominance exists, the mean yield of the progeny of heterozygous plants is the same as their parents, so that changes in the proportions of homozygotes and heterozygotes have no effect on the parent-offspring correlations. Natural selection reduces genetic variation, so that differences between late generation plants tend to be caused more by chance environmental factors than by genetic differences. If genetic variation is eliminated entirely, differences between plants are no longer inherited. Under these conditions parent-offspring correlations will be zero. We thus expect that natural selection tends to lower the parent-offspring correlations of late generation plants.

Natural selection and segregation thus act in opposite directions on the population variances. Where dominance occurs they act in opposite directions on the means and on the parent-offspring correlations. The effect of natural selection depends on the relative magnitudes of environmental and genetic variation, the constancy of the environment from generation to generation, and on the number of generations over which it acts. During the early generations segregation is the more potent force, but it is soon spent, later changes being caused by selection, which, as it reduces genetic variation, also ceases to cause further changes.

By the use of these statistics—population means, variances and parent-offspring correlations—the relative importance of natural selection and segregation can be assessed and the relative values of populations to the plant breeder estimated.

In the first part of this paper the F_2 and F_8 generations of a *Triticum vulgare* cross are so compared; in the second part the effects of selection in these two generations have been compared directly.

2. MATERIAL AND METHODS

The cross chosen for the study was Dreadnought × Cross 7. Dreadnought is a wheat of English origin, and, compared with Cross 7, it has relatively larger grains and more grains per ear, but fewer ears and fewer grains per plant. Cross 7 was bred in New Zealand from Tuscan, a wheat of Mediterranean origin, and White Fife, an old Canadian wheat which originated in eastern Europe. The cross, as the parental and F_1 means show, presents scope for the combination of the higher yield components of both parents to give increased yield, or for the selection

of transgressive segregates with values of yield and its components beyond the parental ranges.

The ancestors of the F_8 were 500 selected F_2 plants. As can be seen from table 7, selection in F_2 may be, in fact, little better than random selection, so that it is considered that the selection of 500 plants from the original F_2 of about 11,000 has had little effect on the bulk derived from them. From F_3 onwards the bulk was grown under normal field conditions, and each year the smaller grains—about one quarter to one third of the total—were sifted out before a sample was taken for sowing.

In 1945 the parents, F_1, F_2 and F_8 populations were grown. For all populations except the F_1 the plots consisted of two rows eight inches apart with 13 seeds four inches apart in each row. The F_1 plots, of which two were grown, consisted of only one row each. The whole material was sown in one block, each population occupying plots at random within the block. In the same block, also in random positions, were parental plots and progenies of plants selected in F_7 which were not used in this particular study. In 1946 the yield and yield components of 136 Cross 7 plants from seven plots, 110 Dreadnought plants from five plots, 25 F_1 plants from two plots, 1846 F_2 plants from 88 plots and 1934 F_8 plants from 91 plots, were determined.

The 75 highest yielding plants were selected from each of the F_2 and F_8 populations. Their progenies were sown in 1946 in two-row plots, rows eight inches apart, with 25 seeds two inches apart in each row. In addition, progenies of 50 randomly selected F_2 and F_8 plants were grown, together with 50 Cross 7 and 48 F_2 plots. Dreadnought was omitted because of its proneness to shed grain. Sufficient seed was available for two replications. Total yield per plot and mean per plant values of yield components of the F_3 and F_9 plots were determined. In addition single plant values of yield and its components of some F_3 and F_9 plots were determined.

The following abbreviations are used:

e = number of ears per plant, or mean number of ears per plant
n = mean number of grains per ear
g = mean weight of one grain (milligrammes)
en = number of grains per plant, or mean number of grains per plant
ng = mean weight of grain per ear (grammes)
eng = weight of grain per plant, or mean weight of grain per plant (grammes)
p = number of plants per plot
peng = weight of grain per plot (grammes)

3. POPULATION STATISTICS

A. Means

The means of the parents and F_1 are given in table 1.

TABLE 1

Means of parents and F_1

	Cross 7	Dread-nought	Difference Cross 7 – Dreadnought	F_1	Difference F_1 – higher parent		p*
					absolute	% increase	
e	4·04	3·39	+0·65	4·30	+ ·26	6·4	·3
n	27·9	28·3	−0·4	31·0	+2·7	9·5	·2–·1
g	51·9	65·0	−13·1**	66·8	+1·8	2·8	·05–·02
en	112	101	+11	129	+17	15·2	·1
ng	1·44	1·74	−0·30*	2·00	+0·26	15·0	·01
eng	5·86	6·61	−0·75	8·70	+2·09	31·6	·01

*p=probability that the mean of the higher parent=the F_1 mean

Statistically significant differences between the parents occur for only two components, *g* and *ng*, but the heterosis of all components in the F_1 shows that this phenotypic similarity masks a high degree of genetic diversity.

TABLE 2

Means of F_2 and F_8

	F_2		F_8		Difference F_8–F_2
	Calculated+	observed	Calculated++	observed	
e	4·01± 1·99	3·80	3·72± ·91	3·83	+0·03
n	29·6± 3·9	27·3	28·1 ± 4·2	28·5	+1·2**
g	62·6± 3·4	58·9	58·5± 2·7	56·9	−2·0**
en	118± 24	104	107± 34	109	+5**
ng	1·80± ·24	1·62	1·59± ·21	1·64	+0·02**
eng	7·47± 1·55	6·16	6·24± 1·93	6·19	+0·03
p		21·99		21·30	−0·69**
peng		136		132	−4·00

+ F_2 calculated=1/4 (P_1+P_2+2F_1)
++ F_8 calculated=1/256 (127P_1+127P_2+2F_1)
** Significant at 1% level

The observed F_2 means (table 2) are consistently lower than the expected F_2 means as calculated from the parents and F_1, but none of the deviations is significant, so that the direct scales of measurement used give adequate estimates of the population means (Mather, 1949). This is confirmed by the absence of correlation between means and variances (*cf.* tables 2 and 3).

In a cross showing dominance, if no effective selection occurs, the mean in each generation after F_2 approaches more closely to the mid-parent mean, the F_8 and mid-parent mean being almost identical.

In this material, the F_2 means of all characters were already close to the mid-parent, so that segregation in succeeding generations would have had little effect on the F_8 population means. Differences between F_2 and F_8 must then be due to selection.

p. The F_2 mean of *p* is significantly higher than the F_8 mean. Engledow and Pal (1934) have reported higher germination in early generations of wheat crosses, while Copeland (1940) has reported higher growth rates of embryos in hybrid than in inbred corn. Some similar effect may account for the high *p* in the F_2 of this cross.

e, *n* and *en*. The mean of *e* is similar in both populations; *n* and *en* are higher in F_8 than in F_2. The number of immediate offspring of any plant is determined by *en*. The main determinant of *en* is *e* (for *e* and *en*, $r = +0{\cdot}93^{**}$; for *n* and *en*, $r = +{\cdot}038^{**}$ in the F_2 population), but *e* is much more subject to environmental variation than *n* (see coefficients of variability below). This explains why natural selection had little effect on *e*, but significantly increased *n* and *en*.

Coefficients of variability

	e	*n*	*g*	*en*	*ng*	*eng*
Cross 7 . .	28	20	8	43	23	44
Dreadnought .	46	22	5	48	15	44

g. The smaller grains were sifted out before sowing. In other *Triticum vulgare* crosses, Copp (unpublished) has shown that mass selection for large grains, as practised in this material, was effective in increasing the mean of *g* in the succeeding generation. In this material, however, such selection, if at all effective, merely counteracted the effects of natural selection, for *g* and *en* are negatively correlated ($r = -0{\cdot}24^{**}$ in the F_8, and $r = -0{\cdot}35^{**}$ in the F_2). Natural selection for *en* therefore resulted in reduced *g*. Had selection for *g* not been practised, the F_8 mean of *g* might have been still lower.

eng. The decrease in *g* is compensated by the increase in *en* so that the mean of *eng* is the same in both the F_2 and F_8.

peng. The lower value of *peng* in F_8 is caused by the lower value of *p* in that population.

To sum up, natural selection for *en* adequately accounts for the changes in the population means between F_2 and F_8. Had these changes been caused by the selection for *eng* in the F_2 from which the F_8 derived, *eng* itself would be higher than in this unselected F_2 (see page 174).

B. Variances

TABLE 3

Variances of parents, F_1, F_2 and F_8

	Cross 7	Dreadnought	F_1	F_2	F_8
e	1·30	2·00	·76	1·93	1·65
n	32	38	42	35	32
g	18	11	40	60	44
en	2258	2319	1067	2059	2117
ng	·11	·07	·18	·16	·14
eng	6·60	8·28	5·84	7·73	6·20

TABLE 4

Variance ratios of parents, F_2 and F_8

	Dreadnought cf. Cross 7	Dreadnought cf. F_2	Cross 7 cf. F_2	F_2 cf. F_8	Dr. cf. F_8	Cr. 7 cf. F_8
e	1·54*	1·04	1·48**	1·17**	1·21*	1·27*
n	1·19	1·08	1·12	1·09**	1·19	1·00
g	1·64**	5·28**	3·35**	1·36**	4·00**	2·44**
en	1·03	1·13	1·10	1·03	1·10	1·07
ng	1·65**	2·45*	1·49**	1·14**	2·00**	1·27*
eng	1·25	1·07	1·17	1·24**	1·34*	1·06

* significant at 10% level ** significant at 2% level

Parents and F_2

In self-fertilised plants the parents and the F_1 generation of crosses are usually considered to be genetically uniform, and are therefore used to estimate the environmental variation present in the experimental area. In fact, the parents may not be devoid of genetic variation, but the ratio of environmental to genetic variance will be much higher in the parents than the F_2. We therefore expect a higher total variance in the F_2 than in the parents and F_1, and that the variances of the two parents will be similar.

The F_2 variances of all components except *en* are higher than the Cross 7 variances, variances of *e*, *g* and *ng* being significantly so (tables 3 and 4). Comparing the F_2 with Dreadnought, however, only *g* and *ng* have lower variances in the Dreadnought population, though no Dreadnought variances are significantly higher than F_2 variances. In addition, the Cross 7 and Dreadnought variances of *e*, *g* and *ng* are significantly different. It is therefore unlikely that the parental variances give a true indication of the ratio of environmental to genetic variances in the segregating populations; obviously there are here interactions between genotype and environment.* Re-scaling could equalise the parental variances, but the other criteria for an adequate scale (see above) would not then be satisfied.

The populations used to estimate the parental variances were small, but the inter-plot variances of those parent plots analysed plant-wise were, except in the case of *e* in Dreadnought, lower than the inter-plant variances of all Dreadnought and Cross 7 plots grown in the experimental area. Apparently the variation of the parents was not over-estimated in the small samples taken.

F_2 and F_8. The F_8 variances of all components except *en* are significantly lower than those of F_2. As has been shown before, segregation increases population variances, selection tends to decrease them. Here, as with the means, the effects of selection outweigh the effects of segregation.

* Similarly high parental variances have been reported by Hutchinson, Panse and Govande (1938).

C. Frequency Distribution

The reduction in variances between F_2 and F_8 may be the result of selective elimination of genotypes at either or both ends of the range. As many of the frequency distributions are not normal, elimination of genotypes from the upper end of the range may have occurred even for those components with a higher F_8 mean. This would reduce the chances of selecting high values. Table 5 compares the percentages of the F_2 and F_8 populations falling above limits set to exclude roughly the top 5% of the population for each character.

TABLE 5

Percentages of F_2 and F_8 above arbitrary limits

	above	F_2	F_8	X^2	p	No. of Plants	
						F_2	F_8
e	5	7·44	7·83	*·21*	<·70	1866	1942
n	37	5·31	6·88	*4·02*	<·05	1846	1934
g	6·70	10·08	5·17	*29·33*	<·01	,,	,,
en	190	4·01	4·65	*·99*	<·40	,,	,,
ng	2·20	5·74	5·69	—	—	,,	,,
eng	10·90	5·20	5·38	*·08*	<·80	,,	,,

There were relatively more F_8 plants in the top fraction for *n*, and less for *g*. No significant changes had occurred for *e* and *en*. Of the plants outside the upper limit for *g*, only 20 of the F_2 and 10 of the F_8 had *eng* higher than 10·90 grammes. Of the plants with *en* above 190, 73 of the F_8 and 64 of the F_2 had yield higher than 10·90. These proportions, together with the effect of selection for *eng* (table 6), show that high *en* is the main cause of high *eng*. The non-significant upward change in *en* has counter-balanced the much larger drop in *g*, so that the proportion of individuals with high values of *eng* remains the same in both populations.

4. SELECTION FOR YIELD

The 75 highest yielding plants were selected from F_2 and F_8.

TABLE 6

Differences between selected and unselected F_2 and F_8

	F_2 Difference Selected — Unselected		F_8 Difference Selected — Unselected	
	absolute	% increase	absolute	% increase
e	2·07**	*49*	2·26**	*54*
n	6·5**	*23*	4·4**	*14*
g	3·6**	*6*	2·3**	*4*
en	98**	*83*	95**	*74*
ng	46**	*27*	·35**	*20*
eng	5·96**	*82*	6·09**	*85*

** significant at 1% level

In the selected fractions of both F_2 and F_8, the means of all yield components were increased (table 6). Of the primary components, *e*, *n* and *g*, the percentage increase was greatest in *e* and least in *g*. Thus selection for *eng* was in effect mainly selection for *e*, the component most affected by environmental variation.

There are two obvious ways in which to judge the effectiveness of selection in the two populations:

1. By a comparison of the parent-offspring correlations, or of the differences between progeny means of selected and unselected plants in each population.

2. By a comparison of the progeny means of plants selected from the two populations.

The first method shows in which population the greater change will be achieved, but does not necessarily show from which population the higher yielding lines will be obtained. It measures the genetic variability of the populations, but disregards their means. The second method fails to show the potentialities for further selective increases, or indeed, whether any advances will be obtained by selection.

TABLE 7

Average plot means of yield and yield components

	Cross 7	F_2	F_3 from unselected F_2	F_3 from F_2 selected for yield	Selected minus unselected	F_9 from unselected F_8	F_9 from F_8 selected for yield	selected minus unselected
e	3·14	3·18	2·97	3·13	+·16	3·04	3·02	−·02
n	28	30	30·15	28·88	−1·27	30·04	30·24	+·20
g	40·3	52·6	48·6	49·5	+·9	47·7	48·6	+·9
en	90	94	88·58	88·97	+·39	91·06	90·80	−·26
ng	1·17	1·54	1·46	1·41	−·05	1·41	1·45	+·04
eng	3·67	4·90	4·30	4·38	+·08	4·29	4·37	+·08
p	26	38	33	36	+3·0	33	35	+2·0
peng	92	184	142	158	+16**	139	153	+14**

The means of the F_3 and F_9 plots were essentially similar (see table 7), so the populations can be compared by the first method.

Selection for *eng* failed to increase *eng* significantly in the succeeding generation. The only significant change was an increase in *peng* in the progenies of selected plants. This increase is due partly to the small non-significant increase in *eng*, but mainly to the increase in *p*. Selection for *eng* was equally ineffective in either population. The parent-offspring correlation of *eng* in the random selections from F_2 is significant, that of the F_8 is not. Judged on this count, selection in F_2 has been slightly more effective than selection in F_8.

Selection for yield in both populations has been a comparative failure. Selection may have been ineffective because environmental variation was high in the F_2 and F_8 populations, or conversely, because genetic variation was low in these populations, though this could hardly

TABLE 8

Rep 1 - Rep 2 and offspring-parent correlation coefficients

		F_9 from unselected F_8	F_9 from selected F_8	F_3 from unselected F_2	F_3 from selected F_2
e	rep 1 - rep 2	·06	·18	·14	·22
	parent offspring	−·06	·16	−·13	·14
n	rep 1 - rep 2	·36**	·32**	·22	·24*
	parent offspring	·15	·23*	·21	·27*
g	rep 1 - rep 2	·79**	·70**	·66**	·83**}+
	parent offspring	·76**	·73**	·73*	·68**}
en	rep 1 - rep 2	·21}+	·21	−·07	·20
	parent offspring	−·26}	·14	·04	·11
ng	rep 1 - rep 2	·50**	·52**	·17	·22
	parent offspring	·22	·33**	·37**	·07
eng	rep 1 - rep 2	·31*	·28*	−·09	·18
	parent offspring	−·12	·07	·27*	−·02

* correlation coefficient significant at 5%
** ,, ,, ,, ,, 1%
}+ significantly different pairs of correlation coefficients 5%

be so for the F_2. It may have failed because environmental variation *per se* in F_3 and F_9 was high.

TABLE 9

Variances of plot means of F_3, F_9 and Cross 7

	F_3 Unselected	F_9 Unselected	F_3 from selected F_2	F_9 from selected F_8	Cross 7
e	·23	·30	·28	·31	·25
n	22	32	35	28	22
g	2·4	2·8	3·0	2·9	1·0
en	320	549	537	554	494
ng	·054	·083	·073	·067	·033
eng	·90	1·16	1·23	1·27	·66

We have shown that the genetic variability of the F_8 is lower than that of F_2. However, if genetic variability of the F_8 population has been reduced to such an extent that further progress by selection is impossible, the variances of the F_9 means should be lower than the variances of the F_3 means, and perhaps similar to Cross 7. In fact they are not, though the variances of the random F_3 plots are unaccountably low (table 9). Presumably, then, lack of genetic variability in the F_2 and F_8 populations is not the cause of the failure of selection.

The relatively high environmental variation among F_2 and F_8 plants must have contributed to the failure of selection, especially as selection

was based mainly on *e*, the primary component most affected by environmental variation. However, we cannot ascribe all the failure to this alone. Correlations between progeny means in the two replications were also relatively low, and seldom significantly different from the parent-offspring correlations (table 8), indicating that environmental variations between F_3 and F_9 plots were considerable.

TABLE 10

Mean intra-plot variances of F_3 and F_9

	F_3	F_9	$F_3 - F_9$	p^*
number of plots	49	50		
e	1·58	1·48	·10	·3
n	81	71	11	·2
g	37	22	15	·01
en	2910	2720	190	·4
ng	·22	·17	·05	·01
eng	7·21	6·12	1·09	·05

p^*=probability that $F_3 = F_9$

For *g*, *ng* and *eng* F_3 intra-plot variances are higher than F_9 intra-plot variances (table 10). For these characters at least, F_2 plants are more heterozygous than F_8 plants. The heterozygosity of F_2 plants will lower the F_2-F_3 correlations only if dominance effects are present in the F_2. This F_2 showed little if any dominance, so that, even though F_2 plants were heterozygous, this would have had little effect on the F_2-F_3 correlations.

One significant effect, an increase in *peng*, resulted from selection for *eng*. This increase is mainly the result of an increase in *p*. The high mortality in the F_3 and F_9 plots was caused by foot-rot. As most of the deaths occurred in the later stages of development, plants adjacent to gaps did not benefit. Consequently, plot means of *eng* were not affected (correlation of *p* and *eng* $= -0{\cdot}05$). The progeny of those plants with the greatest number of ears suffered least deaths, the correlation of parental *e* with progeny *p* being $+0{\cdot}32^{**}$ in both the populations. This correlation is higher than the parent-offspring correlation of *e*. This suggests that those F_2 and F_8 plants with a large *e* possessed some quality, perhaps freedom from seed-borne foot-rot, which enhanced the survival rate of their progeny.

As high environmental variation has been the main cause of the failure of selection, selection for a less variable component, notably *g*, should have been more effective than selection for *eng*. Progenies of high yielding and unselected F_2 and F_8 plants with high values (top 10%) of the individual components are compared with the whole of each population in table 11.

Although some progress was achieved by selection for other components, *g* was the only one which was consistently and significantly

increased. The parent-offspring correlations for the various components show essentially the same result. That for *g* is the only one which is high and consistently significant. However, *g* and *en* were negatively correlated in the F_3 and F_9 populations ($r = -0{\cdot}11$ and $r = -0{\cdot}25$ respectively), and the increase in *g* did not increase *eng*.

TABLE 11

Advances made by selection for components

selected for	selected from: F_2 unselected	p	F_3 high yielding	p	F_8 unselected	p	F_8 high yielding	p
e	+·16	·3	+·09	·5	−·05	>·5	+·16	·3
n	+·42	>·5	+1·80	·1	+0·26	>·5	+1·30	·3
g	+4·7	<·01	+4·8	<·01	+4·8	<·01	+4·2	<·01
en	+1·3	>·5	−0·20	>·5	−6·0	·3	+5·3	·3
ng	−·02	>·5	+0·02	>·5	+·11	·2	+·04	·5

p = probability that this difference = 0

5. DISCUSSION

The evidence presented in the first part of this paper shows that natural selection has been the main force moulding the constitution of the hybrid bulk. In general, natural selection in seed crops favours the higher yielding genotypes (Harlan and Martini, 1938; Laude and Swanson, 1942; for further references see Haldane, 1932). However, the number, rather than the weight, of seeds produced per plant determines which genotypes have the greatest selective value. Where seed weight and seed number are inversely correlated, natural selection may favour lower yielding genotypes. Such a relationship exists in this material and accounts for the relative smallness of the yield increase between F_2 and F_8. Suneson (1949) found that in a mixture of four barley varieties grown for 16 years, the highest yielding genotype was eliminated. He concluded that this was due to the lower competitive ability of the higher yielding variety. Differences in survival rate may have been determined by differences in seed numbers and grain weight, but unfortunately no data on these attributes of the four varieties were given.

In special circumstances, such as those described by Weiss *et al.* (1947) in soya bean where the interaction between seasonal differences and variations in maturity dates was the main determinant of yield variation, natural selection may not eliminate low yielding genotypes.

The similarity of the F_2, F_8 and parental variances, and the low correlations between replications show that environmental variation among single plants and their progenies was high in relation to genetic

variation. Consequently selection for *eng* was ineffective in both the F_2 and F_8 populations. In this material, where dominance effects were virtually absent in F_2, the heterozygosity of F_2 plants had little effect on the effectiveness of selection.

We can fairly say that in these circumstances yield and some yield components behave as unobservable characters, selection based on them being little better than random selection. One component, *g*, could be reliably selected for in single plants. However, as *g* and *en* were negatively correlated, this was of no help in selecting for yield, but suggests that random selection in the early stages of a selection programme may be a safer procedure than selection for relatively unimportant observable characters, which may be adversely linked with yield itself.

Is single plant selection usually as ineffective as it has been in this case? Too few examples have been reported to permit any general conclusions as to its effectiveness. However, these results, and those of some other workers (Christian and Gray, 1941; Panse, 1940; Boyce, Copp and Frankel, 1947) show that it is not universally successful. Selection indices attaching greatest weight to those components with the least environmental variance have been proposed as an improvement (Smith, 1936). Panse (1947) found that these "discriminant functions did not appear to be much superior to straightforward selection on the character itself".

No great difficulty is involved in incorporating tests of effectiveness of selection in a breeding programme. Either bulk plots of the unselected material or random selections will serve as reasonable standards of comparison (cf. Hutchison *et al.*, 1938). If no progress is being made in selection for yield in the early generations of selection, random selection will be preferable to selection based on unimportant ancillary characters.

It might be inferred that a more rigid control of the environment would greatly improve the position. This would increase the effectiveness of selection in any particular environment. However, because the products of selection are grown in a diversity of ever changing environments, rigid control, while increasing the apparent effectiveness of selection, would limit its usefulness.

The present case gives no clear-cut evidence in favour of either the pedigree or bulk method of breeding. However, the increase in the proportion of homozygous plants and the increase in the population mean as a result of natural selection increased the potential worth of the F_8 population. In general, we can expect any changes in the bulked populations to be to the breeders' advantage. The increase in homozygosis confers certain practical advantages in the later stages of selection. The number of generations of single plant selection can be reduced, and the final purification of lines is simplified by the absence of unwanted segregates.

In addition, the bulk method permits selection between crosses

before selection within crosses commences. Inferior crosses can be detected with some degree of precision in bulked yield trials in the F_2 and later generations (Immer, 1941; Harrington, 1940). Consequently, using the bulk method more crosses can be carried, and more plants selected from each cross. The chances of successful selection are therefore enhanced.

Some breeders fear that when using the bulk method they may lose desirable genotypes which would have been preserved by selection in F_2. Akerman and MacKey (1948) discuss this problem, and point out that if the F_2 used to perpetuate the bulk is of sufficient size, such fears are groundless.

It would appear that where only a few genes of importance are segregating, or where speed is essential, the pedigree method is preferable. Where many genes are segregating, or where selection for recessive oligogenes is combined with polygenic selection, the bulked population method makes more economical use of the available facilities, while possibly enhancing the prospects of success.

6. SUMMARY

1. In the wheat cross Dreadnought × Cross 7, a comparison was made between parents, F_1, F_2 and F_8 to determine the changes in yield and yield components which occurred between F_2 and F_8, and to determine the factors responsible for these changes. Means, variances and frequency distributions were compared, and also the parent-offspring correlations of F_2-F_3 and F_8-F_9.

2. Natural selection for high number of grains per plant increased both the number of grains per plant and number of grains per ear. Since the number of grains per plant was inversely correlated with grain weight, the grain weight was reduced.

3. The variances of all components except number of grains per plant decreased between F_2 and F_8. These changes were also the result of natural selection.

4. Except for grain weight, parent-offspring correlations were low and generally non-significant. No consistent differences were observed between F_2-F_3 and F_8-F_9 correlations.

5. Selection for grain weight was successful; selection for yield or the other yield components was not.

6. The relevance of these findings to general plant breeding is discussed.

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THE INHERITANCE OF KERNEL WEIGHT IN A *TRITICUM VULGARE* CROSS

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1. INTRODUCTION

THE study of the inheritance of a complex character, such as yield in wheat, is made difficult by variation caused by differences in the environment, and by the effects of interaction of environment and genotype. Yield per plant may be resolved into a number of sub-characters, or components, which may not be independent in their genetic control (Mather, 1949). The major components, each capable of further resolution, are number of ears per plant, number of kernels per ear, and average weight of a single kernel. Kernel weight has been shown to be the least variable of these components (Smith, 1936).

Estimates of the numbers of genes which determine kernel weight differences in wheat have already been published. Jasnowski (1934, 1935), quoted by Boyce (1948), obtained evidence that certain lines differed by three pairs of genes. Worzella (1942) stated that the inheritance of kernel weight in a number of wheat (*Triticum aestivum* L.) hybrids was multigenic. Boyce (*l.c.*), in a preliminary study, by empirical methods, of the material described in the present paper, concluded that the F_2 and F_3 frequency distributions, and the difference in kernel weight between the two parents, were the result of the action of two or of three major gene pairs.

The plants were grown at the Wheat Research Institute, Lincoln, in 1943/44, but a detailed analysis was not then carried out. Interest in the material was revived following the publication in 1949 of Mather's *Biometrical Genetics*.

2. MATERIAL AND METHODS

The material was one of four crosses used in a preliminary study of the inheritance of kernel weight (Boyce, *l.c.*), comprising the parents, F_1, F_2 and reciprocal F_3 generations of the *Triticum vulgare* cross (India 29, S1556 × Crete 2, S357). Where P_1 (S1556) was used as pollen parent the hybrid material was designated as F_2A or F_3A. The parents were chosen for their high and low kernel weights from a collection of foreign varieties. The purity of the lines had been maintained for several years by sowing grain from ears which had been covered before

anthesis. Each F_3 plot of 52 plants was the progeny of a single F_2 plant. The positions of eight F_1 plots of 26 plants and eight F_2A plots of 52 plants were randomized among the F_3 plots, which were not replicated. Parent plots of 26 plants were grown in random order after every second hybrid plot. Evenness of spacing and depth were obtained by sowing individual grains with a " Woodfield " dibber. The grains were sown two inches deep, with eight inches between the rows of thirteen plants four inches apart.

Plants were harvested individually. For each plant the number of ears (*e*), total number of kernels (*en*), and the yield (*eng*) were recorded; the average number of kernels per ear (*n*) and the average weight of one kernel (*g*) were calculated. Kernel weights for each plant were expressed in milligrams, and ranged from 20 to 61.

Plants with only one ear, or with fewer than forty kernels (about five per cent of those harvested in each population), were omitted from the analysis because average kernel weight would not have been determined with the same accuracy for these plants as for the rest of the material, and because, in many of them, the grain was shrivelled through disease or poor development. The analysis, therefore, is based on healthy plants only.

In studies of quantitative inheritance the amount of material is usually limited, and to obtain the maximum information (from the segregating populations) relatively small parent and F_1 populations are grown. The size of the F_1 is also limited by the work involved in crossing. For any character, all population frequency distributions are commonly presented in one figure. If the same frequency scale were used for all populations it would be difficult to make visual comparisons between them because the differences between the sizes of the popu-

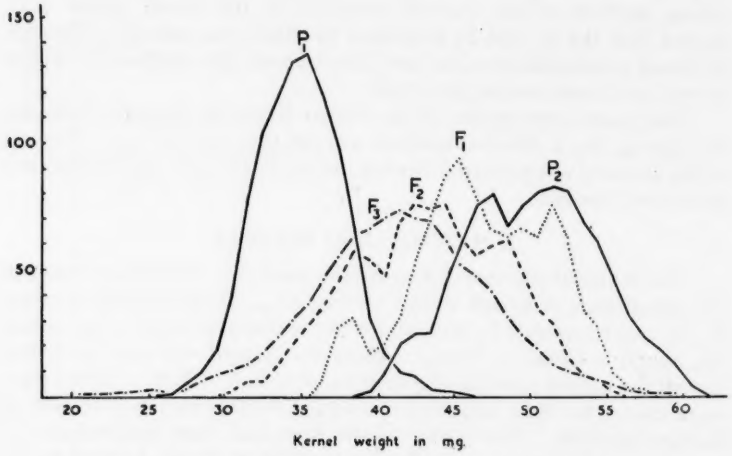

FIG. 1.—Frequency distributions adjusted to equal population size.

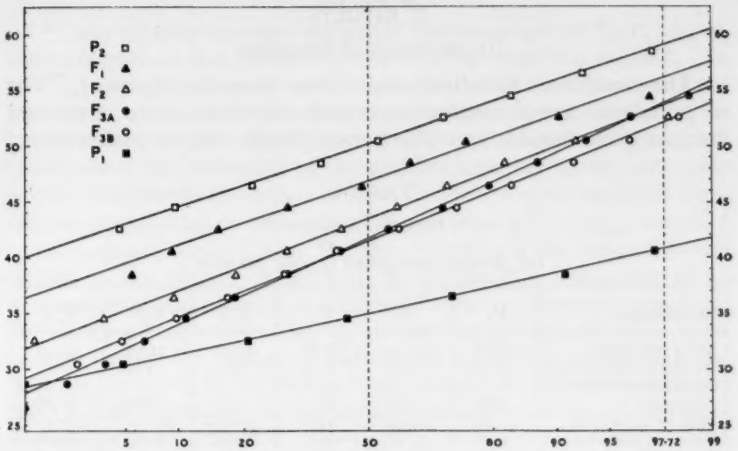

FIG. 2.—Cumulative frequency distributions of kernel weight on probability scale. The mean is located on the ordinate at 50%. The standard deviation is proportional to the slope of the line.

lations are large. A second method, still complicated, in small populations, by the relation between range and population size, would be to adjust the populations to equal total frequencies. In figure 1 a two-term moving average of adjusted kernel weight frequencies has been plotted.

A limitation to these methods of presenting the data is the difficulty experienced in distinguishing and comparing the population means and variances, but on a cumulative probability scale, by which normal distributions are represented by straight lines, the means and variances of the populations are easily compared. In figure 2, which was drawn on probability graph paper, there is, for each population, a point for every second unit of kernel weight over the population range. The numbers of plants in each kernel weight group were accumulated from the lower end of the range and the figures so obtained were expressed as percentages of the total number of plants. These percentages were plotted on the probability scale against the kernel weight, at the mid-points of the class intervals. For example, the percentage with kernel weight 44 or less (which means, in fact, less than 44·50) was plotted at 44·5: if it were plotted at 44·0 and the other points were plotted similarly, the mean, read off at the 50 per cent point, would be one half of the unit of measurement, or 0·5 mg., below the calculated mean. This method of presentation was used by Keller and Li (1949), who did not make this adjustment.

The normal distributions fitting each population are depicted in figure 2 as straight lines fixed by plotting the population mean at 50% probability, and the point twice the standard deviation above the mean at 97·72% probability.

3. RESULTS

(i) *Description of Populations*

The populations had distributions close to normal (figure 2). The six population means and variances and some correlations of the raw data are given in table 1*a*. The parent (S357) with the higher kernel

TABLE 1

Population Statistics

(*a*) *Average kernel weight* (*g*); *mg. per grain*

Generation	P_1	P_2	F_1	F_2A	F_3A	F_3B
Mean per plant	34·96	50·10	46·88	43·41	41·59	41·40
Standard error of mean	0·11	0·13	0·37	0·31	0·106	0·097
Population standard deviation	2·86	4·36	4·54	5·02	6·025	5·281
Variance between plots	*41·48***	*131·98***	*34·80*	*71·29***	*481·90***	*283·48***
Variance within plots	*5·80*	*12·48*	*19·91*	*23·78*	*22·04*	*20·06*
Parent-offspring correlation	0·304	−0·264	—	—	0·737**	0·438**
Number of plots	34	40	8	8	62	64
Number of plants	497	711	143	242	1968	2120

(*b*) *Transformed variate:* $\log^{\frac{1}{2}}(g-25)$

Mean per plant	0·9808	2·3022	2·0591	1·7711	1·6021	1·5925
Standard error of mean	0·0117	0·0098	0·0295	0·0270	0·0095	0·0089
Population standard deviation	0·3095	0·3157	0·3575	0·4317	0·5416	0·4791
Variance between plots	*0·4839***	*0·6412***	*0·1995*	*0·5034***	*3·8999***	*2·2943***
Variance within plots	*0·0681*	*0·0682*	*0·1241*	*0·1769*	*0·1779*	*0·1662*
Parent-offspring correlation	0·251	−0·280	—	—	0·727**	0·407**

weight had a greater variance within plots than the other parent ($F=2{\cdot}15^{**}$), but there was no correlation between plot means and variances in any population. Accordingly, the linear scale of measurement would be considered satisfactory for descriptive purposes. However, for a biometrical analysis of the data, the scale should be such that gene effects appear additive (Mather, 1949, ch. 3) and such that there is no apparent interaction of environment and genotype. The fulfilment of these conditions depends on the positions of the F_2 and F_3 means in relation to those of the parents and F_1, and on the comparison of the variances of the three genetically homogeneous populations, P_1, P_2, and F_1.

From table 1*a*, Mather's $C=4\bar{F}_2-2\bar{F}_1-\bar{P}_1-\bar{P}_2=-5{\cdot}3$

and $D=8\bar{F}_3-2\bar{F}_1-3\bar{P}_1-3\bar{P}_2=-16{\cdot}3$

and using variances within plots $V_C=2{\cdot}16$ and $V_D=1{\cdot}48$, so that C and D (hence $\bar{F}_2$ and $\bar{F}_3$) were significantly lower than would be expected if gene effects were simply additive. In fact, $\bar{F}_3$ was slightly, and significantly, lower than the mid-parent.

(ii) *Scaling*

It may be seen also that the within plot variances of P_1, P_2 and F_1 were unequal, so that neither criterion for the scale was satisfied. In the F_1, more than the scale was at fault, for the plot variances were not homogeneous. A suitable foreshortening scale would equalise the parent variances and improve the relative positions of the means, although it would affect the normality of the distributions. A square root scale was not sufficiently powerful. It was decided to use a modified logarithmic transformation deduced as follows: writing $\overline{G}=\log_{10}(\bar{g}-a)$ for $\bar{g}$ in the expressions for C and D above, where g is the original kernel weight, G the weight on the scale to be found, a a constant to be determined, it was found that C changed sign for a between 25 and 30, and D for a between 20 and 25, so that $\log(g-25)$ seemed a satisfactory variate for analysis. The use of $\log(\bar{g}-a)$ rather than the mean of $\log(g-a)$ in calculating C and D made the approximate determination of a feasible. Unfortunately, on this scale the P_1 variance was higher than that of P_2 ($F=3{\cdot}10^{**}$), and as a compromise a less powerful transformation was needed. Use of $\log(g-10)$ equalised the parent variances but was not satisfactory for the means, $\log^2(g-25)$ gave a smaller variance ratio ($F=1{\cdot}38^{**}$) for the parents than did $\log(g-25)$, and by extrapolation $\log^{\frac{1}{2}}(g-25)$ was thought likely

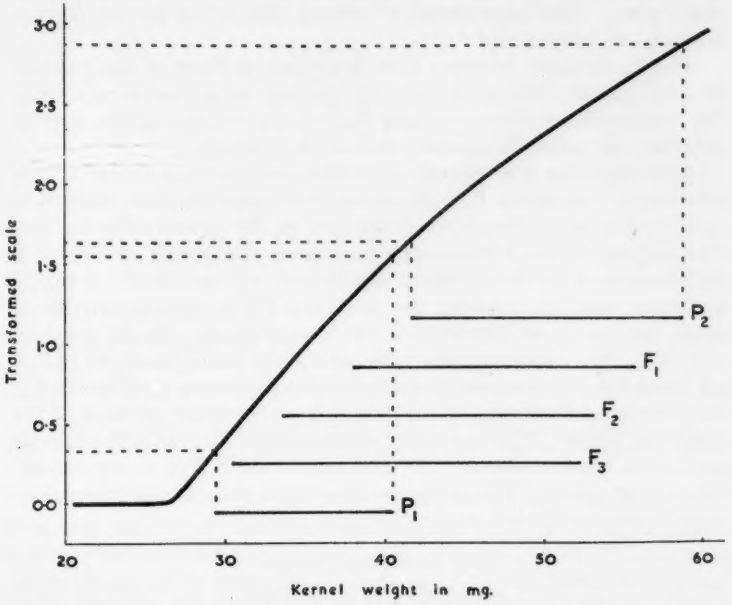

FIG. 3.—Graph of the function $y=\log^{\frac{1}{2}}(g-25)$ for transforming kernel weight figures. Ranges covering 95 per cent of the population distributions. P_1 and P_2 ranges are shown on both scales.

to be adequate. This function is plotted in figure 3. The ratio of parent variances with this transformation was 1·04.

Final calculations of C and D gave $-0{\cdot}317$ and $-1{\cdot}138$, with variances respectively 3·46 and 12·82, which is satisfactory though the F_3 mean is still slightly below the mid-parent. Once it has been decided to use a particular, arbitrary, transformation, its theoretical form is of little interest. It is unlikely that the same transformation would have been appropriate in another year, as in both the preceding and following years the average kernel weights of the parent varieties, grown in the same locality, were significantly higher.

(iii) *Transformed Data*

The statistics derived from the transformed data are shown in table 1*b*.

As already stated, the F_2 and F_3 means are now closer to their expected positions in relation to the means of the parents and F_1. The within-plot variances of the parents have been equalised. The F_1 variances, based on only 143 plants, have not been used in the biometrical analysis because they are still significantly different from those of the parents. The transformation has had no appreciable effect on variance ratios or on the parent-offspring correlations. Except in F_1, the between-plot variances are highly significantly greater than those within plots. The large variance between plots of the parents is attributed to soil heterogeneity.

The F_2 variance between plots is similar to those of the parents, the only genetic differences between F_2 plots being due to sampling. The within-plot variances of the F_2 and F_3, which express genetic variation, are much larger than those of the parents.

Although there is no significant difference in average kernel weight between the reciprocal F_3 populations, their statistics have been presented separately. There are differences in the parent-offspring correlations and in the between-plot variances: the former difference is significant, and the latter almost significant, at 5 per cent. A highly significant difference between the reciprocal F_3's in average number of kernels per ear is not discussed in the present paper. In the previous year six F_2 plots of approximately 60 plants had been grown, three F_2A and three F_2B. A random selection of these plants was used for the F_3; their average kernel weights for A and B were similar to those of the remaining plants. The over-all variance of the F_2A, as of the sample grown, was considerably greater than that of the F_2B ($F = 1{\cdot}37$, significant at 5 per cent). This difference, for which there is no explanation, would have contributed to the difference in F_3.

(iv) *Biometrical Analysis*

(*a*) *Components of Variation*

The second-degree statistics of segregating populations are functions of D, H and E, which are components of variation dependent on the

fixable and the unfixable genetic variation and the environmental variation respectively. The required statistics have the following expression (Mather, *l.c.*):

$V_{F2} = \frac{1}{2}D + \frac{1}{4}H + E_1$, the within plot variance of F_2 plants,

$V_{\overline{F3}} = \frac{1}{2}D + \frac{1}{16}H + E_2$, the variance of F_3 progeny means,

$W_{F2/F3} = \frac{1}{2}D + \frac{1}{8}H$, the covariance of F_2 plants of the previous year with their progeny means,

$\overline{V}_{F3} = \frac{1}{4}D + \frac{1}{8}H + E_1$, the mean variance of the F_3 progenies.

E_1 and E_2 are the non-heritable components of variation for single plants and for means of plots, estimated directly from the non-segregating populations. The least squares calculation of D, H, E_1 and E_2 from these six relations is described by Mather (p. 65). The observed "A" and "B" second-degree statistics and their averages, and the calculated values of D, H, E_1 and E_2 are presented in table 2.

TABLE 2

Observed second degree statistics, derived estimates of genetic and environmental components, and number of effective factors

	A	B	*Combined*
Statistics			
V_{F2}	0·176913	—	*0·262372** / *0·207054*
$V_{\overline{F3}}$	0·131369	0·070628	*0·100999*
$W_{F2/F3}$	0·150489	0·051722	*0·101106*
$\overline{V}_{F3}$	0·181914	0·166120	*0·174017*
E_1	0·068185	0·068139	*0·068162*
E_2	0·021414	0·016159	*0·018786*
V_{VF3}	0·436046	0·532727	*0·484387*
Components of variation			
D	0·280 ± ·129	−0·017 ± ·122	*0·063 ± ·059*
H	−0·072 ± ·413	0·627 ± ·489	*0·628 ± ·238*
E_1	0·081 ± ·035	0·080 ± ·027	*0·074 ± ·013*
E_2	0·009 ± ·035	0·028 ± ·027	*0·025 ± ·013*
$D + \frac{1}{2}H$	0·244 ± ·106	0·296 ± ·140	*0·377 ± ·068*
Number of effective factors			
K_1	1·56 (from D)	0·27 (from H)	*1·39 (from $D + \frac{1}{2}H$)*
K_2	0·009	0·012	*0·018*

* These two figures are estimated from D, H, and E_1, from the "combined" estimates and the "restricted" linkage estimates respectively. There was no "observed" figure.

For the separate "A" and "B" analyses the component E_1 was estimated directly as the within-plot variance of P_2 or P_1: E_2 was estimated as the between-plot variance of P_2 or P_1, divided by the harmonic mean (29·94) of the numbers of plants per F_3 plot. Since no F_2B was grown, for the "B" and "combined" calculations it was necessary to prepare and invert a new matrix of coefficients. This matrix and its inverse are given in table 3.

TABLE 3

Matrix used in "B" and "combined" analyses in calculating D, H, E_1 and E_2 without V_{F2}

$$M=\frac{1}{256}\begin{vmatrix} 144 & 32 & 64 & 128 \\ 32 & 9 & 32 & 16 \\ 64 & 32 & 512 & 0 \\ 128 & 16 & 0 & 512 \end{vmatrix} = \begin{vmatrix} 0{\cdot}5625 & 0{\cdot}125 & 0{\cdot}25 & 0{\cdot}5 \\ 0{\cdot}125 & 0{\cdot}035156 25 & 0{\cdot}125 & 0{\cdot}0625 \\ 0{\cdot}25 & 0{\cdot}125 & 2{\cdot}0 & 0 \\ 0{\cdot}5 & 0{\cdot}0625 & 0 & 2{\cdot}0 \end{vmatrix}$$

$$M^{-1}=\frac{1}{25}\begin{vmatrix} 416 & -1536 & 44 & -56 \\ -1536 & 6656 & -224 & 176 \\ 44 & -224 & 21 & -4 \\ -56 & 176 & -4 & 21 \end{vmatrix} = \begin{vmatrix} 16{\cdot}64 & -61{\cdot}44 & 1{\cdot}76 & -2{\cdot}24 \\ -61{\cdot}44 & 266{\cdot}24 & -8{\cdot}96 & 7{\cdot}04 \\ 1{\cdot}76 & -8{\cdot}96 & 0{\cdot}84 & -0{\cdot}16 \\ -2{\cdot}24 & 7{\cdot}04 & -0{\cdot}16 & 0{\cdot}84 \end{vmatrix}$$

In table 2 it will be seen that the results from the "A" and "B" analyses are different, particularly with respect to the values of *H*. The "combined" analysis gave results very similar to the "B" analysis, but agreeing much more closely with the observed statistics. This similarity may be merely an effect of the omission of F_2 data. The components of variation were not accurately determined. (The "A" standard errors have two degrees of freedom, the others one.) There is therefore little justification for their use in calculating the number of effective factors which differentiate the two parents, or the degree of dominance or potence shown. Mather has suggested that, in view of the negative correlation between *D* and *H* as shown in the two halves of the experiment, an estimate of $D+\frac{1}{2}H$ should be more consistent. The figures given in table 2 support this, and it will be seen that $D+\frac{1}{2}H$ is estimated with the same precision as *D*. The variance of the estimate depends on c_{DD}, c_{DH} and c_{HH} of the inverse matrix.

(*b*) *Test for Linkage*

A modification of Mather's "restricted" analysis, in which the mean variance of F_3 plots is omitted, is available as a test for linkage, though in the present material it would not be sensitive. In the "B" or "combined" analyses there would remain only four statistics involving *D*, *H*, E_1 and E_2, and therefore no residual degrees of freedom. If the calculated "combined" values of *D*, *H*, E_1 and E_2 are used to estimate the second-degree statistics, as in calculating their standard errors, V_{F2} may be estimated also, and the eleven observed "A" and "B" statistics may be compared with these estimates. The sum of squares of the eleven deviations, with seven degrees of freedom, is the total sum of squares in the linkage analysis. Similarly a set of statistics may be estimated from the "restricted" values of *D*, *H*, E_1 and E_2, giving the remaining value of V_{F2} in table 2. Since the four variance components were calculated from only four statistics, the remaining estimates are identical with the observed figures. The sum of squares of the eleven differences between these figures and the observed "A" and "B" statistics is a residual sum of squares; the difference between the total and the residual is the sum of squares, with one degree of freedom, for linkage. The ratio of the linkage and residual *mean* squares was 5·27 ($F_{.05}$, 5·99) which suggests that there is linkage between the

genetic factors influencing kernel weight. However, in the absence of an F_2B this difference in variance cannot be attributed definitely to linkage.

(c) *Number of Effective Factors*

The number of " effective factors " differentiating the parents may be estimated by the use of components of variation and the parent and F_1 means (Mather, *l.c.*, p. 102). If k effective factors have equal effects d, all in one direction, the expected value of $(P_2 - MP)^2$ is k^2d^2, which equals kD, so that k may be estimated as $K_1 = \frac{(P_2 - MP)^2}{D}$ and similarly, if H is known accurately, $K_1 = \frac{(F_1 - MP)^2}{H}$, where P_2 and F_1 represent the corresponding averages and $2MP = P_1 + P_2$. No precise estimate of K_1 can be obtained from this material; the best estimate is $\frac{2(P_2 - MP)^2 + (F_1 - MP)^2}{2D + H}$, which, using $(2D + H)$ from the " combined" analysis, equals 1·39. If, as is likely, the parents differed in many effective factors, an estimate of this order for K_1 would indicate incomplete concentration, the parents having both plus and minus allelomorphs. The data are compatible with a K_1 as large as twelve.

A second estimate, K_2, is derived from the variances of the F_3 plots, as the ratio of the square of the heritable part of the mean variance of the F_3 plots $(\frac{1}{4}D + \frac{1}{8}H)$ to a corrected variance of the plot variances (V_{VF3}, table 2; Mather, *l.c.*, p. 104). This K_2 is expected to be smaller than K_1, except when K_1 is reduced by incomplete concentration, and the relative smallness of the K_2 as shown in table 2 would indicate, if the estimates were reliable and there were in fact many effective factors, that the factors have unequal effects on kernel weight. But the number of effective factors by which the parents differ remains undetermined, and, in consequence, no reliable estimate can be obtained of the immediate limits, or speed, of selective advance.

(d) *Difference between Reciprocal Crosses*

The components of variation estimated from the two halves of the experiment give two different estimates of the genetic difference between the parents. If the discrepancy is real (and the differences in components are certainly not significant) there is a difference between reciprocal crosses. There is a real difference in two of the second-degree statistics, the greater range of the means of the F_3A plots partly explaining their higher covariance with their parent plants. Similarly, the variances of the F_2A progenies in the previous year were greater than those of F_2B.

(e) *Results in Other Years*

The above analysis has been confined to the results obtained in 1943/44, when the difference in kernel weight between the two parents

was $15{\cdot}1 \pm 0{\cdot}1$. In the previous year, under much better growing conditions, the difference was $15{\cdot}9 \pm 0{\cdot}3$, and the higher parent averaged $57{\cdot}3 \pm 0{\cdot}2$ (compared with $50{\cdot}1 \pm 0{\cdot}1$). The relation of the F_1 and F_2 means to the parent means was similar in the two seasons. Three of the F_2 plants were selected for further study, two with average kernel weight slightly greater than P_2 and one much lower than P_1. Their F_3 progenies, grown as part of the main experiment, had average kernel weights much closer to the F_3 mean than the original plants were to their F_2 mean, although the third progeny was again lower than P_1. The progenies of four plants from each of these F_3 plots were grown in the following year, again under good conditions, the F_3 plants in each plot being selected to cover the plot range of kernel weight, and averaging close to the plot average. In this F_4 the kernel weights of the progenies derived from the two high F_2 plants were all nearly as high as the high parent, which averaged $51{\cdot}7 \pm 0{\cdot}2$, but the progenies from the low F_2 plant were significantly above the low parent. The original low F_2 plant was apparently strongly affected by its environment, yet the single-plant selection in F_2 was effective, and much more effective than selection in F_3. This would suggest that the number of effective factors by which the parents differed was not large.

4. DISCUSSION

In this experiment, as in some of those listed by Mather, the components of variation were not estimated precisely, although much material was grown. The design of the experiment did not satisfy all the requirements for biometrical analysis prescribed by Mather; the F_3 plots were not replicated, the F_2 and F_3 plots were twice as large as those of the F_1 and the parents, and the two parents were sown in random order at regular intervals. Only the first of these defects is serious, but it has been pointed out recently (Mather and Vines, unpublished) that more information will be obtained by the growing of a given number of unreplicated F_3 families than from half that number in two replications.

The plots were arranged in eight adjacent blocks, but the parent plots showed no significant block differences. Block differences in the F_3 would be confounded with genetic differences between plots. Replication is desirable, to supply a more precise estimate of the errors of the components of variation and for the test of linkage. Unfortunately, different numbers of plots of the P_1 and F_3 were harvested from each block, so that an analysis with eight " replicates " would not have been justified. The separation of the reciprocal crosses in the present material provided in the second-degree statistics a set of eleven deviations. The standard errors of the " combined " estimates of D, H, E_1 and E_2 derived from the deviations from the " combined " second-degree statistics had only one degree of freedom. If the deviations from the separate "A" and " B " statistics had been used, there would have been seven degrees of freedom available, but the differences be-

tween the reciprocal crosses were such that the test of significance of the components would not have been improved.

It was not possible to find a scale of measurement on which the parent variances would be equalised and the F_3 means would be higher than the mid-parent, though on the scale adopted the F_3 was not significantly below its expectation. The form of the transformation is restricted mathematically only by the condition that it must be continuous and monotonically increasing over the range of expression. In studies of quantitative inheritance there is no reason to expect that one of the " standard " transformations will be the most appropriate. A biometrical analysis of the untransformed data led to conclusions similar to those presented; the transformation made the data conform more closely to the theoretical genetic criteria but its use has not provided more, or more precise, information. It might be suggested that the F_1 mean should have been ignored in testing the scale, since the F_1 plot variances were not uniform, but the mean itself was accurately known, and the F_3 mean was still significantly below expectation based on F_2 and parent means. The most satisfactory effect of the re-scaling, forced to equalise the *within-plot* variances of the parents, was the equalising of the *between-plot* variances of the parents and F_2. Their ratios were changed from 1 : 3·18 : 1·72 to 1 : 1·33 : 1·04.

The difference between the reciprocal crosses affects the estimates of D and H, but not $D+\frac{1}{2}H$. It has been stated that the difference may be attributable to a difference in the F_2 of the previous year, but the origin of this difference is unknown. It is unfortunate that no F_2B was grown with the F_3's. The original F_2 difference might have been a consequence of differential incidence of seed-borne (and hence " maternally inherited ") disease in the first F_1 plants.

In this material the weighting of the second-degree statistics was not conducive to the improvement of the estimates. Weighting the observations E_1 and E_2 heavily did not increase the accuracy of estimation, which suggests that the re-scaling had not removed all interaction of genotype with environment. A disadvantage of weighting is that the required matrix of coefficients is not one of the standard forms, and is not symmetrical. The standard matrices are relatively easy to invert by Fisher's method, particularly if the equations set up are cleared of fractions; the terms of the inverse matrices are found as fractions with a common denominator which is, apart from powers of two, the value of the determinant of the original matrix. Thus in Mather's table 12 (p. 61) c_{HH} is exactly $\frac{8704}{93}$, which equals 93·591398, and not 93·591441 as given.

Kernel weight was shown by Smith (1936) to be the most effective yield component for differentiating between certain varieties of wheat. The present parents were chosen because they differed widely in average kernel weight: 98·6 per cent of the parent plants could be classified correctly on kernel weight alone, and about half of the remainder were

classified correctly if number of kernels and number of ears were also taken into account.

Boyce (1948) showed that the shape of the F_2 distribution of kernel weight could be explained by the segregation of two partially dominant genes, but she was unable to find any satisfactory explanation for the F_3 distribution. No doubt it would be impossible to get a statistically satisfactory fit in this large F_3 (Haldane, 1949). Formal estimates of number of genes, or effective factors, are unreliable, but they are valuable in indicating whether the effects of genes are equal and in the same direction. The data suggest that the effective factors influencing kernel weight are possibly linked, have unequal effects, and do not all act in the same direction.

Effectiveness of *selection* for yield components may be less than for total yield (Boyce *et al.*, 1947). In the material of Palmer (1952), although selection for kernel weight was effective, it had no effect on total yield, because kernel weight was negatively correlated with number of kernels per plant. The present F_4 data show that a line with kernel weight as high as the high parent is recoverable, and that it may be possible to select a line as low as the low parent. This information about the limits of selection is more definite than that obtained from the biometrical analysis.

The application of Mather's methods of analysis has not been successful. The variances and covariances of the segregating populations did not have the general relationships expected on simple genetic reasoning: the F_2/F_3 covariance was relatively high in the "A" material and low in the "B". There are several possible explanations. The difference between parental genotypes is fixed, but would have a different expression in another environment. Some mistakes would have been made in counting, weighing and computing, and these would have increased the error variances even if no bias were introduced. Non-heritable variation, sampling variation and residual genic interaction would have lowered the precision of the analysis. Sampling variation would be expected to be small in this large material, and non-heritable variation appears to have been adjusted by the re-scaling, leaving residual genic interaction as the most probable explanation for the lack of success. There is no suggestion that any other method of analysis would have given more information.

5. SUMMARY

1. The inheritance of kernel weight was studied in the first three hybrid generations of a varietal cross in *Triticum vulgare*, using the biometrical methods developed by Mather.

2. A transformation was derived by which the data satisfied Mather's two criteria for an adequate scale of measurement. The empirical method of obtaining the transformation, by means of the formulae for the tests of a scale, has been explained.

3. The advantages of presenting a set of frequency distributions on probability graph paper have been discussed.

4. Although the material was large the estimates of the genetic and environmental components of variation were not sufficiently precise to enable any definite conclusions to be drawn regarding the number of effective factors segregating, their linkage relationships or the possible limits to selection. It has been suggested that residual genic interaction has affected the precision of the estimates.

5. There were unexplained differences between the distributions of the reciprocal F_3 progeny means, which produced differences between the separate estimates of the components of variation.

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TOLERANCE TO HOMOGRAFTS, TWIN DIAGNOSIS, AND THE FREEMARTIN CONDITION IN CATTLE

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1. INTRODUCTION

THE present paper is a sequel to our first published enquiry (Anderson, Billingham, Lampkin, and Medawar, 1951) into the use of skin grafting as a means for distinguishing between one-egg and two-egg twins in cattle. Experience with laboratory mammals has shown that skin does not long survive transplantation between animals having any lesser degree of affinity than may be achieved by upwards of a dozen consecutive generations of strict inbreeding; failing this, skin so grafted will elicit an immune response from its host—the " homograft reaction "—and succumb to it after a period which rarely exceeds two weeks. Since two-egg twins are no more closely related to each other genetically than are ordinary full siblings, it was expected that skin grafts exchanged between them would be no exception to the rule. On the other hand, skin grafts exchanged between one-egg twins should, like autografts, survive indefinitely.

In practice it turned out otherwise. Skin exchanged between what were presumed to be monozygotic twins did indeed survive indefinitely; but the great majority of dizygotic twins also proved to be tolerant to grafts of each other's skin, even when they were of unlike sex. Their tolerance was thrown into high relief by the fact that grafts exchanged

between ordinary siblings provoked homograft reactions of great violence, and no such graft lived longer than 15 days.

Owen (1945; Owen, Davis and Morgan, 1946) has already demonstrated the tolerance of two-egg twin cattle to genetically foreign tissue by other means, for each member of such a pair contains not only the red blood cells that derive from its own zygote division lineage, but also those that derive from the zygote lineage of the other member of the pair. In effect, therefore, the twins have the same blood groups, at least during their youth. Since red cells are continuously manufactured anew throughout life, this can only mean that the two-egg twin calf contains living cells of two zygote lineages of different genotypes and is therefore a genetical chimaera. It has presumably become so as a result of the mutual exchange of red cell precursors during embryonic life that is made possible by the embryos' having confluent blood circulations. Another presumed consequence of the synchorial condition is the notorious fact that the female member of approximately 90 per cent of two-egg twin pairs of unlike sex is sterile.

The failure of our attempts to identify two-egg twin calves by mutual exchange of grafts led us to pursue and extend our work on the following lines:

(i) An extension of our series of graft interchanges between cattle classified by orthodox methods as two-egg twins. We have already shown that not all two-egg twins are fully tolerant to grafts of each other's skin, and we were interested in finding out whether the proportion of twins in which exchanged grafts were not acceptable was approximately the same as the proportion of normal or almost normal females to be found in twin pairs of unlike sex. If the synchorial condition is a necessary prerequisite both for mutual graft tolerance and for the infertility of the female in twin pairs of unlike sex, then the proportions should be fairly similar.

(ii) An extension of our " control " experiments to include exchanges of skin between dam and offspring of both single and two-egg twin birth. These complete the grafting tests that are necessary to demonstrate the uniquely anomalous behaviour of two-egg twins towards genetically foreign tissue.

(iii) An examination of the possibility of using other specialised methods of grafting for distinguishing between one-egg and two-egg twins.

2. METHODS

The practice of transplanting ear skin grafts to the " withers " has continued to give satisfactory results, and only one technical improvement has been made. Instead of relying upon a Hessian truss around the thorax (1951, plate 1, figs. 7, 8, 9) to protect the grafts and bring adequate perpendicular pressure to bear upon them, we now pass a strip of 3 in. wide Lastonet bandage round the entire thorax, the free ends being brought together under tension and secured to each other temporarily with Michel's suture clips. The Lastonet bandage is then stuck down to the skin by a thin film of Copydex. (Lastonet is an open mesh unmedicated elastic bandage, and " Copydex " a watery solution of rubber latex.) The Lastonet prevents side

slip, and, being applied under tension, provides all the perpendicular pressure that is necessary. The immediate graft dressings—tulle gras, a layer of surgical gauze, and a thick rectangular pad of cotton wool now so shaped as to overlap the graft-bearing area by about an inch all round—remain unchanged, and the dressings are completed by fitting a loose Hessian blanket to give added protection.

This variant of our technique has given particularly satisfactory results.

3. RESULTS

(i) *Further Grafting Experiments on two-egg Twins*

The results of our new tests on the transplantation of skin from one member of a two-egg twin pair to the other are summarised by table 1. They relate to 10 pairs of female twins, 5 pairs of male twins, and one group of triplets consisting of a male and two (almost certainly) monozygotic females. The animals we used were not of course always known with complete certainty to be two-egg twins, though there was no reason to question the accuracy of the diagnosis. Twins of dubious classification were not used.

We have already published the results of exchanging grafts reciprocally between ten pairs of two-egg twins of which three pairs were of unlike sex (1951, tables 3 and 5). These earlier results had made it clear that twins are by no means necessarily symmetrical in their responses: grafts transplanted from one twin to the other might be tolerated during the entire period of observation in spite of the fact that grafts of the reciprocal transplantation were eventually destroyed. In our present experiments we used the limited time at our disposal by grafting the majority of our twin pairs " one-way " only—each member being either a donor or a recipient, but not both—instead of grafting a smaller number reciprocally. Nothing is lost by this procedure if there is no internal correlation between the responses of the twin pairs. But there must surely be *some* such correlation, even if it falls far short of complete symmetry of response, and if this is so, our sampling net is cast so much the wider by the procedure we have actually adopted.

Either three or four grafts were transplanted from one twin to the other. Two animals, DZ 28A and DZ 29A, were grafted on a second occasion because the grafts originally transplanted to them showed signs of weakness and inflammation. The behaviour of the second set of grafts showed that these pathological changes were the consequence of faulty healing and not of a homograft reaction.

Taken as a whole, our present results and those already published give evidence on 42 animals which received grafts from their respective two-egg twins. All animals displayed a high degree of tolerance to homografts from their twins, for no graft lived for less than 70 days after transplantation; only six animals showed a degree of intolerance that ultimately led to complete graft breakdown. We may therefore take it that 36 animals in 42, about 86%, were completely tolerant within the time limit of the experiment, which on only two occasions was less than 100 days.

TABLE 1

Donor	Recipient	Sire	Dam	At operation: Age (days)	At operation: Wt. (lbs.)	Observation period (days)	Notes
D 4B	D 4A	Sh.	Sh. × H.	240	408	*131*	
D 10B	D 10A	H.	Fr.	126	148	*131*	
D 12B	D 12A	,,	Sh.	119	180	*131*	
D 13B	D 13A	,,	Sh.	97	172	*114*	Breakdown almost complete at 74 days; see also Table 2B and plate 1, figs. 1, 2
D 14B	D 14A	,,	A.	70	130	*131*	See also table 2B
DZ 18A	DZ 18B	,,	Sh.	209	325	*131*	
DZ 19B	DZ 19A	,,	A.	223	338	*95*	Grafts normal at 40th day, broken down by 95th day
DZ20B	DZ20A	,,	Sh.	174	267	*131*	
DZ 21A	DZ 21B	,,	Sh.	102	160	*91*	See also tables 2B, 3B
DZ 21B	DZ 21A				159	*129*	
DZ 24A	DZ 24B	,,	Fr.	93	176	*119*	Breakdown in progress in 24A at 119 days; cf. tables 3A, 3B
DZ 24B	DZ 24A				171		
DZ 27B	DZ 27A	,,	Fr. cross	96	147	*131*	See also table 3B
DZ 28B	DZ 28A	,,	Fr. cross	94	159	*36+95*	See also tables 2B, 3B
DZ 28A	DZ 28B	,,		130	204	*95*	
DZ 29B	DZ 29A	,,	Sh.	91	191	*64+67*	
DZ 29A	DZ 29B			155	269	*67*	
DZ 30B	DZ 30A	,,	Sh.	65	120	*131*	
DZ 31B	DZ 31A	,,	Sh.	65	112	*131*	
B 61	MZ 61A	Fr.	Fr.	52	109	*103*	Triplets. See also table 3A, and plate 1, figs. 6 and 7
MZ 61A	B 61				116		
B 61	MZ 61B				114		
MZ 61B	B 61				116		

TABLE 1.—Transplantation of grafts between twin pairs classified as two-egg. All homografts remained normal to outward appearance over the stated period of observation unless the contrary is stated. Entries for weight, age, observation period, etc., relate to the recipient animals entered in each row. Animals numbered with Z in the prefix are female; all others are male.

Note.—Abbreviations of Breed Names: Sh., Shorthorn; H., Hereford; Fr., Friesian; A., Ayrshire.

(ii) *Transplantation of skin from mothers to their calves*

We have already described the consequences of exchanging skin grafts between (*a*) unrelated cattle, individually of twin birth (1951, table 1); (*b*) a set of four pedigree Ayrshire cows, individually of separate birth (1951, table 1); and (*c*) full siblings of separate birth (1951, table 4). Every such homograft was destroyed by the 15th day following its transplantation, and the majority were completely necrotic by the 9th day.

These control experiments do not quite suffice to show that the behaviour of two-egg twins is unique, because anomalous tolerance to homografts might conceivably extend to skin transplanted from dam to calf. We therefore extended our survey to include homografts transplanted (*a*) from dam to calf of single birth, and vice versa; (*b*) from dam to calves of two-egg twin birth; the reciprocal transplantations could not be done. The results are summarised by tables 2A and 2B respectively.

Homografts transplanted from dam to calf of single birth survive no longer than they do when transplanted between full siblings of separate

TABLE 2A

Donor	Recipient	Sire	Dam	At operation: Age (days)	At operation: Wt. (lbs.)	Homograft survival time (days)
Swain's Whim (dam)	Abgro Ruby (calf)	A.	A.	519	780	$>7 \leqslant 15$
Abgro Ruby (calf)	Swain's Whim (dam)	A.	A.	1989	1200	<7
Staverton M. (dam)	P4 (calf)	Fr.	Fr.	292	498	$>7<10$
P4 (calf)	Staverton M. (dam)	Fr.	Fr.	1283	1300	Grafts accidentally lost
Swain's Violet (dam)	AF4 (calf)	Fr.	A.	54	124	$>7 \leqslant 15$
AF4 (calf)	Swain's Violet (dam)	A.	A.	2087	1150	<7

TABLE 2A.—Reciprocal exchange of grafts between dam and calf of single birth. The three calves were females.

birth. Evidently the calves are in no appreciable degree tolerant to their mothers' cells. Owing to a technical fault, only two of the three dam/calf pairs gave evidence of the fate of homografts in the reciprocal transplantation, i.e. from calves to dam. It is noteworthy that in these two pairs the homografts on the dam broke down very rapidly indeed, the process being complete by the 7th day. Histological examination of the graft at the 7th day showed that there had been no proliferation of the skin epithelium; the graft vessels were grossly dilated and engorged with red cells, and their endothelial linings had long since been disrupted; and cellular infiltration of the dermis by leucocytes was comparatively slight, no doubt because of the precocious breakdown of the blood vessels (cf. plate 1, fig. 8). In common laboratory animals, this combination of properties is distinctive of " immune " breakdown, i.e. of the behaviour of homografts transplanted to animals that have been immunised by an earlier grafting of skin from the same donor. The evidence, though slight, hints at the possibility that the mother had been in some degree immunised against (and had therefore become specially intolerant to) her offspring's cells. It cannot be said whether or not the immunisation was concomitant with the pregnancy of the calves studied here, for all the mothers were multiparous.

TABLE 2B

Donor (Dam)	Recipients (calves)	Sire	At operation: Age (days)	At operation: Wt. (lbs.)	Homograft survival time (days)	See also:
Sh.	D 13A	H.	133	228	>20	Table 1; plate 1, figs. 4, 5, 6
	D 13B			231	$>13<16$	
A.	D 14A	H.	106	196	$>13<16$	Table 1
	D 14B			164	$>11<13$	
Sh.	DZ 21A	H.	221	333	$>10 \leqslant 14$	Tables 1, 3B
	DZ 21B			332	$\leqslant 10$	
Fr. cross	DZ 28A	H.	158	256	$>10 \leqslant 14$	Tables 1, 3B
	DZ 28B			249	<10	

TABLE 2B.—Transplantation of grafts from dam to twin calves classified as dizygotic. The first two pairs of calves are males, the others females.

Table 2B shows that homografts transplanted from a dam to each of her two-egg twin calves also broke down. This fact settles the main question at issue by showing that grafts exchanged between dam and calves of two-egg twin birth are not exempt from the consequences of transplantation immunity. Two other facts, however, require an explanation: (i) although the homografts eventually broke down, they lived longer (cf. plate 1, fig. 3) than homografts transplanted from dam to calves of single birth (table 2A) or between full siblings of separate birth (1951, table 4); and (ii) that the homografts survived for different lengths of time on the two members of the twin pair (contrast plate 1, fig. 3 with plate 1, figs. 4, 5). These apparent anomalies will be discussed in section 4 (ii).

(iii) *Other skin grafting methods for distinguishing between one-egg and two-egg twins*

It has now been clearly shown that the mutual exchange of skin grafts between twin cattle cannot be relied upon to distinguish their modes of origin. Grafts exchanged between two-egg twins *sometimes* break down, but very much more often they do not. Any incompatibility that may be revealed is, moreover, of very low degree, for grafts which break down at all usually live for two or three months before finally doing so.

We have therefore tested a method of twin diagnosis based on different principles. The two-egg twin calf, although a genetical chimaera, must surely consist of cells that are *predominantly* of one genotype; in particular, the skin of such a calf contains cells of which upwards of 99% presumably derive from one zygote lineage, and it may be that only a very few red cells and leucocytes derive from the other. Grafts

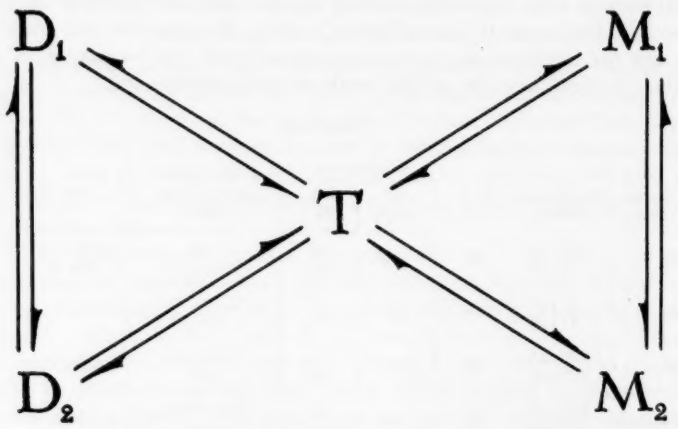


FIG. 1.—The style of grafting operation used in tests 1 and 2 (table 3A). Each arrow represents the transplantation of three grafts. D1, D2 and M1, M2 are two-egg and one-egg twins respectively; T is a test animal unrelated to either. Each animal received three autografts in addition to the 12 homografts indicated by arrows.

from two-egg twins should therefore be antigenically distinguishable, and if grafts from them are transplanted on the same occasion to a third and unrelated " test animal ", their survival times should differ perceptibly (Medawar, 1945; Dempster and Lennox, 1951; Billingham and Medawar, 1951).

With this possibility in mind, we therefore conducted two tests in the grafting pattern illustrated by text-fig. 1 and a third test in the pattern illustrated by text-fig. 2. Each test should make it possible

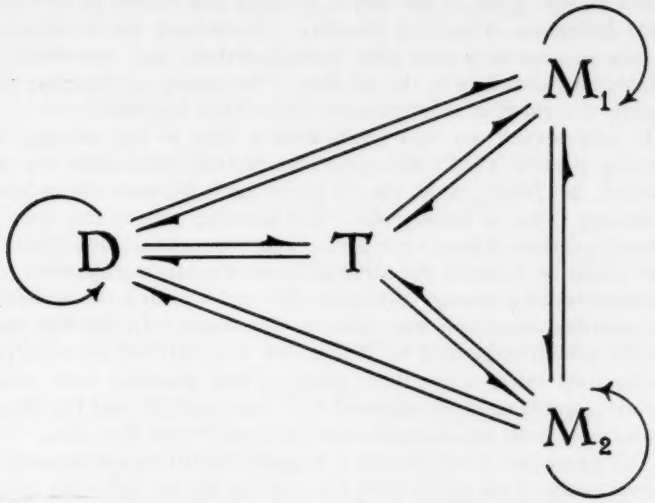


FIG. 2.—The pattern of grafting operation adopted for test 3 (table 3A). D, M1, M2 are triplets of which M1, M2 are almost certainly one-egg twins. The principle of the test is the same as that illustrated by text-fig. 1.

(*a*) to decide whether grafts from two-egg twins D1 and D2 elicit reactions of different intensity after transplantation to an unrelated test animal T; (*b*) conversely, to decide whether grafts from a test animal T elicit distinguishable reactions from D1 and D2; (*c*) to compare two-egg twins in each such respect with one-egg twins M1 and M2; and (*d*) to compare differences of genuinely immunological origin with any which, being revealed in grafts from the same donor to the same recipient, must have arisen in some other way. No appreciable differences of this last sort were in fact revealed.

Interpretation was based almost wholly upon the histological analysis of biopsy specimens, for the outward appearance of homografts can be most misleading. In the first test, biopsy specimens were taken at the 7th and 10th days; in the second test, at the 9th day; and in the third test at the 9th and 12th days.

Animal D in test 3 was twin brother to the monozygotic female twins M1 and M2: as table 1 shows, each animal was tolerant for at least 103 days to skin from its two-egg twin (see plate 1, figs. 6, 7).

On the other hand, table 1 shows that one of the two-egg twins in test 2, DZ 24A, eventually reacted against grafts from its twin DZ 24B.

In summary of an exhaustive series of comparisons between grafts, it can be said that the only distinct differences that were revealed were between the grafts from T on D1 and on D2 in test 2, and between the grafts from T on D and on M1 (or M2) in test 3. Otherwise the results were negative: differences to be expected theoretically were not revealed. There is no reason to doubt that this is because the reaction against foreign grafts in the cow is so rapid and violent as to obscure subtle differences of reaction intensity. Breakdown was invariably in progress so soon as 7 days after transplantation, and was almost invariably long complete by the 9th day. The results are therefore more properly described as uninformative rather than negative.

It later turned out that grafts from a dam to her two-egg twin offspring (section 3 (ii)) did quite unexpectedly what tests 1-3 were expected, but failed, to do, viz. to discriminate between the responses of two-egg twins to homografts. Not knowing this at the time, we adopted a variant of tests 1-3 in an attempt to accentuate any difference there might be between the survival times of grafts transplanted from the members of a two-egg twin pair (D1 and D2) to a test animal T. The modified operation was done in two stages. In the first stage, 4 grafts were transplanted to T from one only (D1) of the twin pair. Twelve days later, when these grafts of first planting were wholly necrotic, 4 grafts were transplanted to T from both D1 and D2. Biopsy-specimens of both sets of grafts were removed seven days later.

This procedure was expected to magnify the difference between the survival times of the grafts from D1 and D2 for the following reason. Although D1 and D2 certainly share many of their antigens in common, it was to be expected that D1 should contain some antigens absent from (or present in negligible amounts in) D2. The immunity developed in T as a consequence of the earlier grafting of skin from D1 must therefore be directed more strongly against D1 than against D2.

Three sets of tests were done. The results showed that a high degree of immunity was directed against grafts from both D1 and D2, for grafts from both donors showed the accelerated breakdown, suppressed epithelial proliferation, precocious vascular stagnation and relatively sparse leucocyte infiltration that are characteristic of homografts transplanted to animals already immunised against them (plate 1, fig. 8; contrast plate 1, figs. 9, 10). In all three tests, the grafts from D1 showed these characteristics more strongly. The distinction was not sharp enough to make the basis of a workable routine test, but sharp enough to justify the hypothesis that D1 and D2 do not share all their antigens in common. The mistake was to choose as a test animal a cow too distantly related to either twin: the parallax of the comparison was too fine.

Information relating to the animals used in the two classes of tests described in this section is contained in table 3.

TABLE 3A

Test	Animal No.	Sire	Dam	At operation: Age (days)	At operation: Wt. (lbs.)	Notes
1	T=P10	Fr.	Fr.	140	296	Test animal; plate 1, figs.9,10
1	M1=MZ 56A	H.	Sh.	100	169	One-egg twins
1	M2=MZ 56B	H.	Sh.	100	180	One-egg twins
1	D1=DZ 21A	H.	Sh.	102	159	Two-egg twins
1	D2=DZ 21B	H.	Sh.	102	160	Two-egg twins
2	T=FA4	A.	Fr.	108	218	Test animal
2	M1=MZ 53A	Fr.	A.	162	225	One-egg twins
2	M2=MZ 53B	Fr.	A.	162	217	One-egg twins
2	D1=DZ 24A	H.	Fr.	93	171	Two-egg twins
2	D2=DZ 24B	H.	Fr.	93	176	Two-egg twins
3	T=AF3	Fr.	A.	150	277	Test animal
3	M1=MZ 61A	Fr.	Fr.	52	109	One-egg twins; Triplets
3	M2=MZ 61B	Fr.	Fr.	52	114	One-egg twins; Triplets
3	D=B61	Fr.	Fr.	52	116	One-egg twins; Triplets

TABLE 3A.—Information about the animals used in the experiments illustrated by text-fig. 1 (Tests 1 and 2) and by text-fig. 2 (Test 3). Animal B61 was male; the others are females.

TABLE 3B

Test	Animal No.	Sire	Dam	At operation: Age (days)	At operation: Wt. (lbs.)	Notes
1	T=JF4	Fr.	Jersey	232	370	Test animal
1	D1=DZ 28A	H.	Fr. cross	115	206	Two-egg twins
1	D2=DZ 28B	H.	Fr. cross	115	204	Two-egg twins
2	T=P5	Fr.	Fr.	280	571	Test animal; see plate 1, fig. 8
2	D1=DZ 24A	H.	Fr.	162	278	Two-egg twins
2	D2=DZ 24B	H.	Fr.	162	291	Two-egg twins
3	T=P6	Fr.	Fr.	274	501	Test animal
3	D1=DZ 27A	H.	Fr. cross	117	181	Two-egg twins
3	D3=DZ 27B	H.	Fr. cross	117	187	Two-egg twins

TABLE 3B.—Information about the animals used in the second series of tests described in section (iii), in which skin was transplanted from both members of a pair of supposedly two-egg twins to an unrelated test animal that had already been immunised against skin from only one of them. All animals were females.

4. DISCUSSION

The results described here confirm and extend those of our earlier report. All cattle we tested were in some degree tolerant to homografts from their two-egg twins, even when the twins were of unlike sex; the majority were, within the terms of our experiments, completely tolerant.

The mutual exchange of skin grafts therefore failed, in the majority of trials, to distinguish between one-egg and two-egg twins. Tolerance, however, is not symmetrical: if D1 and D2 are dizygotic twins, grafts from D1 may survive on D2 although those from D2 eventually break down after transplantation to D1. The toleration of genetically dissimilar grafts is peculiar, in cattle, to two-egg twins; it extends neither to ordinary siblings nor to grafts exchanged between dam and calf.

(i) *Anomalous graft tolerance in relation to the freemartin condition*

The work of Owen (*loc. cit.*) justifies the strong presumption that tolerance to homografts exchanged between two-egg twins is a consequence of the same peculiarity of embryonic development as that which leads to sexual abnormality in the female member of two-egg twin pairs of unlike sex: the anastomosis of the foetal circulations. If this is so, the proportion of animals in which vascular anastomosis is so complete (or has happened so early?) as to lead to female sterility (the freemartin state) should be correlated with the proportion of animals in which it has made possible the development of tolerance to homografts. Exact correspondence is not to be expected: sexual abnormality and homograft tolerance may provide measures of different sensitivity: there are degrees of both, and there is no knowing what degree of abnormality corresponds to any particular degree of tolerance.

Of 42 individual cattle of either sex that received homografts from their respective two-egg twins, 36 (*i.e.* about 86%) proved to be completely tolerant within the time limits of observation. Swett, Matthews and Graves (1940) give 11 out of 12 as the approximate proportion of freemartins to be found in the female members of dizygotic twin pairs of unlike sex—*i.e.* about 92% of such females have achieved a degree of vascular anastomosis that is made evident by some degree of infertility. The correspondence is close enough to be regarded as strong evidence that homograft tolerance and female sterility share at least one necessary causal condition in common.

(ii) *Asymmetry of reaction to skin homografts in dizygotic twins*

The experiments of section 3 (iii) gave weak evidence that when skin is transplanted from an unrelated test animal to each member of a two-egg twin pair, the two members respond to the grafts by reactions of slightly different intensity. When, however, grafts are transplanted from a dam to each of her two-egg twin offspring, the distinction becomes very clear indeed, as may be seen from the entries in table 2B.

The distinction may be more conspicuous merely because homografts from dam to two-egg twin offspring last longer than homografts between unrelated animals. They also last longer than grafts transplanted between full siblings or from dam to calves of separate birth. Such a prolongation of survival is to be expected, because each individual, being unresponsive to its twin's antigens as well as to its own, is clearly unresponsive to a wider range of antigens than a calf of single

FIG. 1.—Appearance of homograft from D 13B 74 days after transplantation to its two-egg twin D 13A. Breakdown is very nearly complete, and only traces of much eroded surface epithelium remain. The vascular system of the graft is no longer functional. Contrast with plate 1, fig. 2, showing a graft from the same donor to the same recipient 8 days after transplantation. ×58.

FIG. 2.—Appearance of homograft from D 13B 8 days after transplantation to its two-egg twin D 13A. The graft shows some degree of traumatic inflammation, but no specific pathological change. Note the hyperplastic condition of the epidermis. Contrast with plate 1, fig. 1. ×58.

FIG. 3.—Illustrating the perfectly normal appearance of a homograft 20 days after its transplantation from a dam to one of its two-egg twin calves (D 13A). New hairs have begun to form and glandular epithelium is well differentiated. Contrast with plate 1, figs. 4, 5, which illustrate the grafts transplanted from the dam to the other member of the twin pair. ×58.

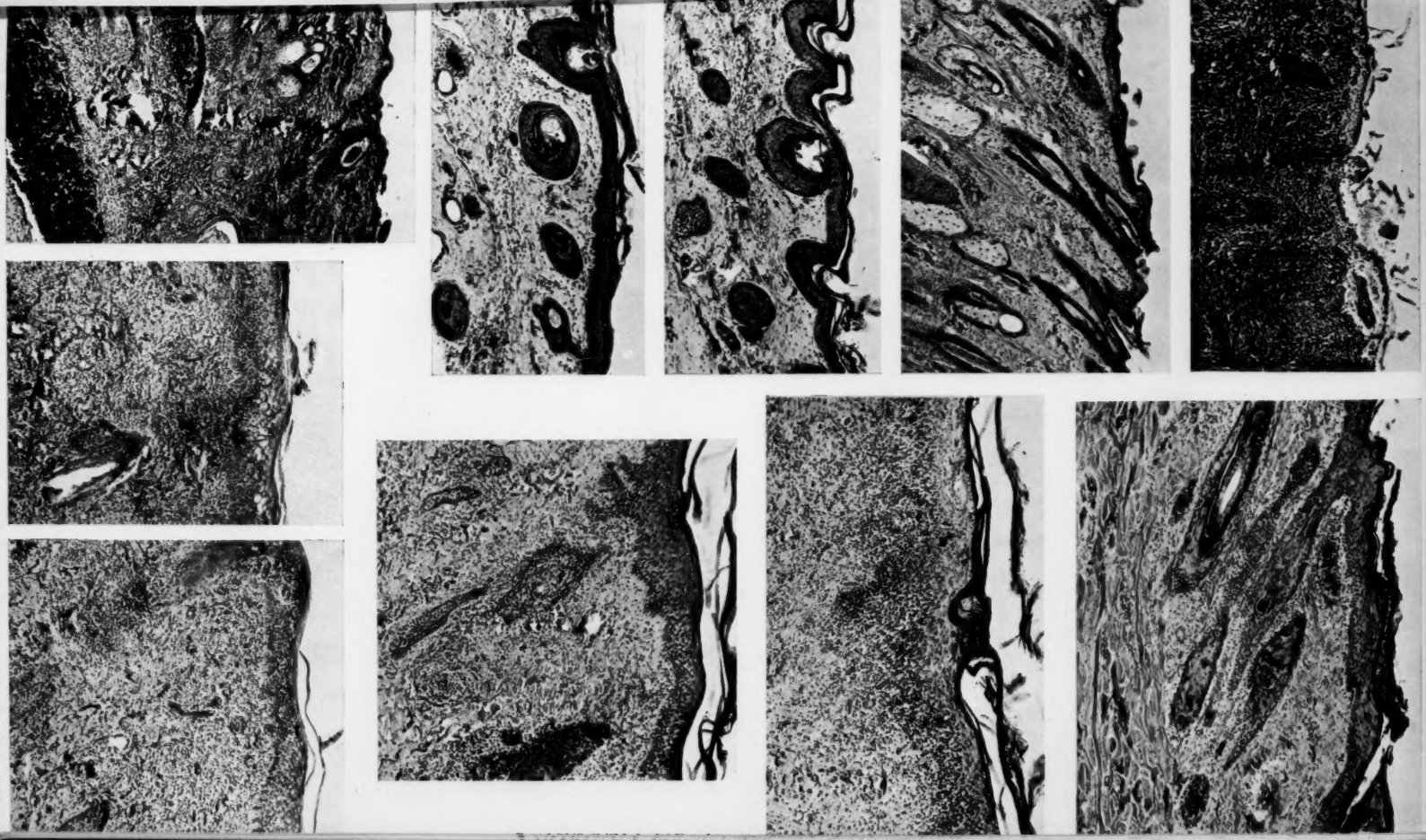
FIGS. 4, 5.—Contrast with fig. 3: illustrating the beginnings of breakdown in a homograft 11 days after its transplantation from a dam to the other (D 13B) of its dizygotic twin calves. The cellular reaction in the graft dermis is not very intense (contrast, for example, figs. 9, 10) but erosion of the follicle and surface epithelium has clearly begun. Breakdown was complete by the 16th day. Figs. 3, 4, 5 illustrate both the prolongation of survival to be expected in grafts transplanted from dam to dizygotic twin calves and the differences between the responses of the calves themselves. ×58.

FIGS. 6, 7.—Transverse sections through an 8-day homograft transplanted from the male (B 61) to a female (MZ 61B) member of a set of triplets (table 1). The graft is in every respect autograft-like, and continued to be so over the period of observation (103 days). ×58.

FIG. 8.—Illustrating "immune" breakdown: a homograft from DZ 24A seven days after transplantation to a recipient, P_5, which had already received and reacted against grafts from the same donor source (table 3B). Breakdown is complete: note the enormously dilated and now stagnant blood vessels and the complete absence of any sign of epithelial proliferation. Contrast with figs. 9, 10. ×58.

FIGS. 9, 10.—Illustrating an "acute" homograft reaction: a graft from DZ 21A seven days after transplantation to an unrelated test animal P10 (table 3A). Gross cellular infiltration of the dermis; maceration and vacuolation of the strongly hyperplastic epidermis. Contrast with the "immune" type of breakdown illustrated by fig. 8. ×58.

All histological specimens have been stained with Ehrlich's haemotoxylin and eosin.



birth. (Dissimilar triplets should be still less responsive than dissimilar twins, and so on; it would not be surprising if grafts from a dam to any one of a group of dissimilar quintuplets lasted for several months.) What *is* surprising is not that two-egg twins should react weakly to skin from their mother, but that they should react differently. By the ordinary luck of segregation, a dam may well contain antigens present in neither of her two-egg twin offspring; but an antigen conferred upon either one is, according to our interpretation, effectively conferred upon the other as well; for the one twin does not in fact react against antigens which are present in the other though absent from itself. In short, if twins are tolerant to grafts from each other, they should be to an equal degree intolerant to grafts from their mother.

There may be two sorts of explanation for the fact that grafts from the dam survive for different lengths of time. The twins may be expected to differ in respect of genetical factors that influence their capacity to react against foreign tissue. Alternatively, it may be that foetal cell interchange is rarely if ever a symmetrical process, so that one member of the pair becomes more or less tolerant than the other. The second possibility must surely account for part of the inequality of reaction, for it is a well established empirical fact that the responses of twins to grafts from each other do indeed differ widely wherever some degree of intolerance allows it to be seen that they differ at all.

We have not yet studied the responses of one-egg twins to grafts from their mothers, though it is in the highest degree unlikely that they would differ to any appreciable degree. Nor have we done sufficient experiments to say whether the grafting of skin from a dam to her two-egg twins is a uniformly reliable method of revealing their two-egg origin, though of all the methods we have so far tested, it is the only one that looks hopeful.

5. SUMMARY

1. Thirty-six out of 42 cattle of two-egg twin birth were found to be completely tolerant to skin homografts transplanted from their respective twins. Homografts between full siblings or from dam to offspring of separate or twin birth are not tolerated.

2. All two-egg twins show some degree of tolerance to homografts transplanted from one to the other, and the degree of tolerance is widely variable.

3. Two-egg twins are not symmetrical in their responses: grafts from one twin to the other may be tolerated although grafts of the reciprocal transplantation are eventually destroyed.

4. The proportion of cattle of two-egg twin birth that show complete tolerance to grafts from their respective twins corresponds closely with the proportion of females in two-egg twin pairs of unlike sex that reveal some degree of infertility or sexual abnormality. It is therefore argued that homograft tolerance and the freemartin state share at least

one necessary causal condition in common. This condition is presumed to be the confluence of the foetal circulations of cattle twins.

5. Mutual graft exchange cannot be used as a method of distinguishing one-egg from two-egg twins in cattle.

6. In each of four trials, grafts from a dam elicited significantly different reactions after transplantation to each of her two-egg twin calves. Later experiments may reveal that this asymmetry of reponse provides the basis of a reliable method of identifying twins of two-egg origin.

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LINKAGE RELATIONS OF THE BLOOD GROUP GENES OF MAN

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Introduction.—The results to date are here given of a search for linkage between the genes for human blood groups, for phenyl thiocarbamide tasting and for sex. The calculations are based on 487 families with two or more children. The blood grouping tests were done between 1938 and 1951 by this Unit or by its parent, the Galton Laboratory Serum Unit, established by Professor R. A. Fisher, with the support of the Rockefeller Foundation. Many of the families have appeared in papers from the two Units, but with those groups alone recorded which were appropriate to the particular study.

The *u* statistics of Fisher (1935*a*, 1935*b*) have been applied to the pedigrees, which have been treated as "data for the double backcross". Thus, we have scored the "certain" families of Finney (1940) which fall into the "mating types" 20 and 21. Since these pedigrees can be analysed in so many ways, we hope that it may be possible to make available the complete collection, including one-child families.

The earlier families could of course be tested for relatively few characters and consequently contribute much less to the general amount of information than do the hundred or so families which have been tested for the nine blood group systems now known. The point may be laboured thus: five of the recent families, taken at random, afford a total κ of 85: had they been counted in 1939 for *ABO*, *MN*, *P*, P.T.C. and sex alone, the total κ would have been 14.

Conclusions.—Our results are shown in the table; they disclose no likely linkages though the amount of information in many of the comparisons is small enough to leave plenty of room for future detection. In making so many counts it is to be expected that some of them will by chance approach significance at the 1 in 20 level, and in two comparisons, that between *P* and *Duffy* and that between *Duffy* and *Kidd*, $S(\lambda)$ is just greater than $1{\cdot}64\sqrt{S(\kappa)}$. In both these counts information is small and addition of the "doubtful" families (Finney, 1940) makes $S(\lambda)$ less than $1{\cdot}64\sqrt{S(\kappa)}$.

Discussion.—Our results, where they overlap, support the findings of previous workers. Many of the past investigations have been confined to the testing of pairs of sibs. The most numerous *family* blood group results which have been analysed for linkage are: 166 families tested

Linkage scores of the "certain" families amongst the 487; the upper figure showing $S(\kappa)$, the lower $S(\lambda)$

	P.T.C.	*Kidd*	*Duffy*	*Lewis*	*Kell*	*Luth.*	*Rh*	*P*	*MNS*	*ABO*
Sex	27 −1	22 +4	48 +4	96 −6	85 −5	32 +8	665 −43	110 +6	718 0	515 +3
ABO	24 +4	28 0	51 −3	71 +15	27 −1	41 −5	313 +7	54 +2	402 +16	
MNS	38 −10	31 −1	62 +6	49 +1	65 −5	40 −6	481 +27	97 −15		
P	5 −3	— —	10 +6	20 0	6 −2	7 −1	95 −1			
Rh	19 +1	23 +5	59 −5	65 +3	66 −12	35 −3				
Lutheran	2 −2	3 +1	16 −4	4 0	— —					
Kell	4 +2	6 0	8 −2	10 −2						
Lewis	5 −3	2 +2	18 0							
Duffy	7 −1	3 +3								
Kidd	8 +2									

for *ABO* and *MN* by Landsteiner and Levine (1928) and analysed by Wiener (1932), 131 families analysed for *ABO* and *MN* by Wiener and Vaisberg (1931), 66 families analysed for *ABO* and *MN* by Zieve, Wiener and Fries (1936), 30 families tested for *ABO*, *MN*, P.T.C. and sex by Boyd and Boyd (1941) and analysed by Finney (1941) and 60 families tested for *ABO*, *MN* and *Rh* (*Rh* positive and negative) by Landsteiner and Wiener (1941) and analysed, with 40 more families, by Wiener and Sonn (1943).

Mohr (1951*a* and *b*) has produced very strong evidence of linkage between the *Lutheran* genes and the *Lewis* genes. This was first detected by the sib method of Penrose (1946) and, after the testing of 17 complete families selected for possessing the antigen Lu^a, it was shown too by the *u* statistics. By neither method of analysis do our own families give any hint of the linkage, but this is one of the comparisons for which our information is slight.

That linkage between two genes is not detectable when "information" is adequate does not necessarily mean that they are not on the same chromosome, but it does mean that if they are on the same chromosome they must be so far apart that the linkage is not likely to be detected until a third gene is found within demonstrable linkage distance of both. Bearing this qualification in mind, it is clear from

our results that none of the genes for the 9 blood group systems, or for P.T.C. tasting is partially sex-linked. It is also clear that the genes for *ABO*, *MNS* and *Rh* are not linked to each other or to the genes for *P*, *Lutheran*, *Kell*, *Lewis*, *Duffy*, *Kidd* or P.T.C. The information is somewhat inadequate in most of the remaining 21 of the 55 comparisons.

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THE EFFECTS OF NATURAL SELECTION ON BODY COLOUR IN THE LAND SNAIL *CEPAEA NEMORALIS*

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1. INTRODUCTION

It has been shown in earlier papers (Cain and Sheppard, 1950; Sheppard, 1951) that, contrary to the opinion of other workers, variation in the colour and banding patterns of the very polymorphic land snail *Cepaea nemoralis* (L.) is not random. It is controlled by natural selection. Two main types of selection are acting; one is physiological, producing here a condition of stable polymorphism, and the other predatory, determining the actual ratio of phenotypes in each colony according to the aspect of the background on which it lives. Lamotte (1950) has also shown, independently, that predatory selection by thrushes results in a differential elimination of banding-patterns.

In the course of our earlier work it was noticed that there appeared to be a correlation between the darkness of the background and the shade of body colour of the snails living on it. This paper describes an investigation of this correlation. It is found that here again variation is dominated by natural selection and is not random.

2. METHODS

Sampling.—Random samples were taken from nineteen collecting stations, nine of which were from different parts of the same colony. At each station, all the living adult snails seen were taken.

Localities.—The collecting stations were chosen so that the samples were drawn from different types of background. These were as follows:

Rockley Colony (Wiltshire):

Station 1. Beechwood with some green herbage.
2. Dense beechwood.
3. Beechwood with grass.
4. Open downland adjacent to beechwood.
5. Open downland 50 yards away from beechwood.
6. Beech hedge (neglected and very dense, principally with bare earth and drifts of beech leaves beneath it).
7. Open downland.
8. Beech copse, dense.
9. Beechwood, moderately open, but with little green vegetation.

Ashbury (Berkshire): Dense beechwood.

Rough Down (Wiltshire): Open downland.

Sheepstead Hurst (Berkshire): Rough green herbage (meadowsweet, comfrey, willowherb, nettles, etc.).

Frilford (Berkshire): As Sheepstead Hurst.
Hinksey Hill (Berkshire): As Sheepstead Hurst.
Cumnor Hill (foot) (Berkshire): Rough green herbage (principally garden weeds).
Stretham (Cambridgeshire): Hedgerow with much grass.
Botley hedge (Berkshire): Hedgerow with green herbage.
Wytham Lane (Berkshire): Hedgerow with green herbage.
Marley Wood (Berkshire): Open mixed deciduous wood with much light brown leaf litter.

Earlier collections from Rough Down, and Rockley 1, 2 and 3 combined, were reported on by Cain and Sheppard (1950).

Scoring.—The shell colours and banding patterns were classified on the system already described by Cain and Sheppard (1950).

The body colour in *C. nemoralis* is predominantly some shade of grey, deepening to black in some individuals, and almost absent in others. In certain individuals there is a tinge of yellow throughout, and in a few a suffusion of a reddish-chocolate colour. It was found possible to ignore these tinges and match each animal against the series of 21 neutral shades given at the left-hand edge of each plate in the colour atlas of Villalobos (1947), which ranges from black (0) to white (20).

As the shade is not uniform over the body of each snail, the forehead was chosen for matching, because it gives a good indication of the general body shade. All scoring was done with individuals as nearly as possible fully expanded, since the shade varies with the degree of expansion, especially in the lightest individuals.

Pigment.—The cytological basis of the body shade was investigated with material fixed in formal-calcium (Baker, 1944) and sectioned on the freezing microtome, gelatine-embedding being unnecessary. The histochemical methods used are given by Lison (1936).

Environmental effects.—A number of snails, mostly immature, were kept together in the same container on the same diet for some months (30 May to 8 Sep. 1951) and were scored for body shade at intervals, in order to determine how far differences in body shade could be ascribed to environmental influences. The container was a wooden tub covered with a sheet of glass, and one-quarter filled with damp earth and turf. It was placed in an unheated shed. The humidity in it was high at all times. Lighting was diffuse daylight from a nearby window; its intensity at the bottom of the tub was at most as high as in a dense beechwood at the same time of day. The food used was lettuce, and corn-flakes put out on damp filter-paper which was also eaten.

3. RESULTS

The results of scoring for colour of shell, banding of shell, and shade of body are given for each collecting station in tables 1-3. The correlations between the percentage of yellow shells and greenness of the background, and between the percentage of effectively unbanded shells and uniformity of the background which were shown previously (Cain and Sheppard, 1950) hold good for these samples of which all but four are from new localities.

TABLE 1

Relation between phenotypes of shell and body shade

Locality	Body Shade	Yellow				Light Fawn		Fawn				Pink		Red			Brown			Totals
		023(45)	0(23)00	00300	00000	00300	00000	12345	00345	00300	00000	00300	00000	(12345)	00300	00000	(12345)	00300	00000	
Rockley 1	2																	1		1
	3										1					1				2
	4			1							1								3	5
	5			2								1					1		2	6
	6			2	1								1						3	7
	T.			**5**	**1**						**2**	**1**	**1**			**1**	**1**	**1**	**8**	**21**
Rockley 2	2			1									2		1				1	5
	3			2	3							1	2		2				6	16
	4			1	6						3	1	3		4			1	8	27
	5			4	4						3	1	8		15				14	49
	6			5	3				1			1	2	1	2				9	24
	7			1	1							1			1				3	7
	8			2											1			1	3	7
	9				1						1		1							3
	11																		1	1
	T.			**16**	**18**					**1**	**7**	**5**	**18**		**1**	**26**		**2**	**45**	**139**
Rockley 3	2			1							1		1		1				2	2
	3			2									1		1				2	7
	4			3	4					1	2		2						5	17
	5			2	7					2	1	1	1		1				7	22
	6				3		1				6								2	12
	7			1									2						1	4
	8			1															2	3
	9				1														2	3
	T.			**10**	**15**		**1**			**3**	**10**	**1**	**6**		**3**				**21**	**70**
Rockley 4	2																		1	1
	3				1														2	3
	4				2					1	1								2	6
	5			2	1					2	2								5	12
	6			4	5					1	2		4						11	27
	7			7	2					2	5	3	2						10	31
	8			3	5					1									5	14
	9				2						2								3	7
	10			1	1														1	3
	11				1						1								1	3
	13				1															1
	16																		1	1
	17				1															1
	T.			**17**	**22**					**7**	**13**	**3**	**6**						**42**	**110**

TABLE 1—*continued.*

Locality	Body Shade	Yellow				Light Fawn		Fawn				Pink		Red			Brown			Totals
		023(45)	0(23)00	00300	00000	00300	00000	12345	00345	00300	00000	00300	00000	(12345)	00300	00000	(12345)	00300	00000	
Rockley 5	3																		1	1
	4			3	1					1									2	7
	5			2	3						3	1	1			1			7	18
	6			9	8					1	4		2						12	36
	7			10	9					3	1	1	2					2	11	39
	8			7	9					1	4	1	1						12	35
	9			7	6					1	3		1						6	24
	10			3	3					1									2	9
	11				3						1								3	7
	12		1	4	1														2	8
	13			1	3														1	5
	14			1	2														2	5
	15			1							1								1	3
	16																		1	1
	17				3															3
	T.		1	48	51					8	17	3	7			1		2	63	201
Rockley 6	3			1						1				1						3
	4			1	1	1						3		1	1				3	11
	5			1	3	1				1	1	3			1				11	22
	6			1	3							2	1	2	2				15	26
	7				1					1		2							4	8
	8				1							1							4	6
	9									1									5	6
	13																		1	1
	T.			4	9	2				4	1	11	1		4	4			43	83
Rockley 7	5	1																		1
	6					1							1						3	5
	7			3	3														3	9
	8			2	1					2	1								4	10
	9				1						1						1			3
	10				1								1						1	3
	13			1																1
	14				1															1
	17			1																1
	T.	1		7	7	1				2	2		2					1	11	34
Rockley 8	3			1															1	2
	4				1						1	2		1			1		5	11
	5			2	1					1		1	3			1			2	11
	6												1						2	3
	7			1															1	2
	8																		1	1
	T.			4	2					1	1	3	4	1		1		1	12	30
Rockley 9	3			1									1		1					3
	4			2						1								2	2	7
	5				1			1		2									5	9
	6									2	1							1	1	5
	7																		1	1
	T.			3	1			1		5	1		1		1			3	9	25
Ashbury	2																		1	1
	4							1			1		1						1	4
	5							1		1	1								1	4
	6																		1	1
	7							1											1	2
	T.							3		1	2		1						5	12

TABLE 2

Relation between phenotypes of shell and body shade

Locality	Body Shade	Yellow									Fawn									Pink					Brown			Totals
		12345	123(45)	02345	10345	12045	00345	10305	00300	00000	12345	123(45)	(12)3(45)	(123)(45)	02345	02(345)	0(2345)	00345	00300	00000	12345	1(23)45	(12345)	02345	00300	00000	00(345)	00300	00000	
Frilford	3											1																	1
	5	1																					1						2
	6										2																		2
	8								1																				1
	9	1							1										1										3
	10				1						1										1							1	4
	13	1																											1
	T.	**3**			**1**				**2**		**3**	**1**							**1**		**1**							**1**	**14**
Rough Down	5									2																1	2		5
	6									4																2	3		9
	7						1		2	5														2			8		18
	8								1	6					1				3							1	1	4	17
	9								1	4								1						1			1	2	10
	10									3							1										4	8
	11			1					1	3																			5
	12									2									1										3
	13																										1		1
	14									1															1				2
	16									1																			1
	T.			**1**			**1**		**5**	**31**					**1**	**1**	**1**		**4**					**3**	**1**	**1**	**5**	**24**	**79**
Sheepstead Hurst	2	1																		1									1
	3																			1									1
	4	2							1		1				1													1	6
	5	3	1								2											2						2	10
	6	4									2		2	1									1					1	11
	7								1	1																			2
	8	1									1				1														3
	9										2																		2
	10	2							1																				3
	11	1									1																		2
	12										2																		2
	13																										1		1
	14																			1									1
	15																							1					1
	T.	**14**	**1**						**3**	**1**	**11**		**2**	**1**	**2**				**2**			**2**		**1**	**1**			**5**	**46**
Stretham	4	2					2														1								5
	5	1						1																					2
	6	2			3	1			1												1								8
	7	4																											4
	8	4			1				1																				6
	9				1																								1
	14							1																					1
	T.	**13**			**5**	**1**	**2**	**2**	**2**												**2**								**27**
Hinksey Hill	5	1																											1
	6	1			1																								2
	7	5	1	1					1	1									1										10
	8	3		1	1		1		1	1	1																		9
	9	3																											3
	10	5									1																		6
	11										1																		1
	12			1			1																						2
	13	1								1																			2
	15								1		1																		2
	16	2							1																				3
	17										1																		1
	T.	**21**	**1**	**3**	**2**		**2**		**4**	**3**	**5**								**1**										**42**

TABLE 3

Locality	Body Shade	Yellow													Light Fawn				
		12345	123(45)	(12345)	(12)3(45)	(123)(45)	02345	10345	00345	003(45)	00045	000(45)	00340	00300	00000	12345	123(45)	10345	12340	00345
Cumnor Hill Foot	6	1																		
	8	1																		
	9																			
	12	1													1					
	14														1					
	T.	3													2					
Botley Hedge	4						1		2											
	5						2		2											
	6	1			1	1														
	7	2					1						1		1					
	8	4					1													
	9	2	1				1													
	10	1					1													
	11	2						1												
	12	2																		
	13	1					1													
	14	1					2													
	15	1							1											
	T.	17	1		1	1	10	1	5				1		1					
Wytham Lane	4							1												
	5	4										1						1	1	
	6	1	1												1	2				
	7	2									2				1	1				
	8	1								1						2				
	9	1						1	1											
	10	1														1				
	11	1															1			
	12	2																		
	13	1													1					
	14																			
	15																			
	16																			
	18																			
	T.	14	1					2	1	1	2	1			3	6	1	1	1	
Marley Wood	5	1																		1
	6								1											
	7	1	1								1									
	8							1						2						
	9																			
	10	1																		
	12													1		1				
	13	1																		
	14	1																		
	15																			
	16					1	1													
	17			1												1				
	18																			
	T.	5	1	1		1	1	1	1		1			3		2				1

Relation between phenotypes of shell and body shade

Locality		Fawn									Pink					Brown	
	Body Shade	12345	123(45)	02345	02(345)	12045	00345	00045	00300	00000	12345	1(23)45	123(45)	(123)(45)	00300	00000	**Totals**
Cumnor Hill Foot	*6*																1
	8																1
	9	2															2
	12																2
	14																1
	T.	**2**															**7**
Botley Hedge	*4*															2	5
	5	1															5
	6	1															4
	7	1														3	9
	8															2	7
	9															1	5
	10															2	4
	11																3
	12															1	3
	13	1														1	4
	14															2	5
	15															1	3
	T.	**4**														**15**	**57**
Wytham Lane	*4*																1
	5																7
	6	1						1								1	8
	7	1				1		1								2	11
	8	1															5
	9															1	4
	10															1	3
	11																2
	12																2
	13															2	4
	14	2														1	3
	15															1	1
	16															1	1
	18															1	1
	T.	**5**				**1**		**2**								**11**	**53**
Marley Wood	*5*															1	3
	6	1											1				3
	7	3		1					1								8
	8		2														5
	9	1		1							1				1		4
	10	2					1									1	5
	12		1		1												4
	13	1							1	1							4
	14									1							2
	15	2															2
	16													1			3
	17																2
	18											1					1
	T.	**10**	**3**	**2**	**1**		**1**		**2**	**2**	**1**	**1**	**1**	**1**	**1**	**2**	**46**

TABLE 4

Dependence of body shade on age and environment (1951)

Phenotype of individual shell in order of increasing lightness of body		Body Shade					
		30 May	15 June	19 July	7 Aug.	24 Aug.	8 Sept.
Yellow	00000	4^3	5^3	6^4	5^4	5^4	5^4
,,	00300	4^2	4^2	4^3	5^4	4^4	5^4
Pink	00000	4^3	5^3	5^4	5^4	4^4	4^4
Yellow	,,	5^3	7^3	4^4	5^4	4^4	4^4
,,	00300	—	5^2	5^3	6^4	6^4	5^4
Pink	00000	—	5^2	6^2	5^2	5^2	5^3
Brown	,,	5^3	5^3	5^4	5^4	4^4	3^4
,,	,,	5^3	5^3	4^3	4^4	5^4	4^4
,,	,,	5^3	5^3	6^4	6^4	5^4	5^4
,,	,,	5^3	4^3	5^4	5^4	6^4	5^4
Yellow	00300	6^3	6^3	6^4	5^4	5^4	5^4
,,	,,	6^4	5^4	6^4	5^4	5^4	4^4
,,	00345	6^2	7^2	6^4	5^4	6^4	5^4
Brown	00000	6^2	6^2	6^2	6^3	—	—
,,	,,	6^2	5^2	5^4	5^4	4^4	4^4
,,	,,	—	9^2	7^3	7^3	7^4	7^4
Yellow	00300	—	10^4	13^4	11^4	11^4	11^4
,,	00000	11^4	10^4	9^4	9^4	10^4	10^4
Brown	,,	12^4	12^4	—	—	—	—
,,	,,	13^2	10^2	9^4	11^4	10^4	10^4
,,	,,	13^2	11^2	10^2	9^2	11^2	12^3
Yellow	(12)345	—	14^4	11^4	13^4	16^4	16^4
,,	,,	—	15^4	15^4	14^4	16^4	16^4
,,	12345	16^4	14^4	—	—	—	—

The indices show the size of each animal at scoring, on the scale
1=quarter-grown 2=half-grown 3=three-quarter-grown 4=adult

It will be seen from the tables that there is a strong tendency for individuals from colonies with a low percentage of yellow shells to be darker than those from populations with a high one. In fact, on darker backgrounds there are fewer yellow shells, and fewer light-bodied individuals.

Microscopical examination shows that the colour of the body is due almost entirely to cells containing pigment granules. Most of these cells lie just below the epidermis and are typical melanophores, being branched, and crowded with minute granules of approximately uniform size. Others lie deeper in the body wall, are less branched, and contain large aggregations of pigments (plate 1). In dark-bodied animals the melanophores are abundant and full of black or dark brown granules. In pale-bodied animals there are very few cells with pigment, and the pigment itself may be a pale red-brown. The granules are argentaffine, and bleach easily with bromine and hydrogen peroxide. They dissolve slowly in concentrated sodium hydroxide solution, not at all in acids. The pigment is therefore a melanin (Lison, 1936, p. 249).

The results of the lighting experiment described under 'environmental effects' are shown in table 4. There is neither a systematic darkening nor lightening with increasing age, nor a change towards uniformity of body shade. There is some variation in the scores, especially those of the lighter individuals. This is due almost entirely to different attitudes of the buccal mass, and slight contractions of the body, which have most effect in the lighter individuals. The maximum variation so produced is 4 units in the very light snails (range 9-18) and decreases with increasing darkness, being 3 for range 3-6.

4. DISCUSSION

Correlation between body shade, shell colour, and background

A comparison of sections taken from snails previously scored for body shade shows that the scores are good indicators of the degree of pigmentation and can be used to represent it in a statistical analysis (plate 1). If the body shade is affected directly by the genes controlling shell pattern and colour, then there will of necessity be a correlation between these three variables, all of which must be considered in the analysis. Shell colour (x) was scored as either yellow (1) or not yellow (0); body shade (y) was scored on the scale of neutral shades (0-20), and the number of bands (z) was recorded (0-5). Then a regression between stations of body shade or shell colour is equivalent to a weighted regression on the proportion of yellows. An analysis of variance and covariance of y on x and z was carried out.

When variation within stations totalled over all localities was considered, it was found that the bivariate regression on x and z was just significant at the 5 per cent level.

$$y = \bar{y} + 0{\cdot}263(x - \bar{x}) - 0{\cdot}140(z - \bar{z})$$

There was no significant difference between the regressions calculated separately for each site. Neither of the single-variate regressions on x and z was significant.

The regression of y on x between stations (fig. 1) was highly significant ($P<0{\cdot}01$). The inclusion of z in the regression did not reduce the residual sum of squares significantly. The mean square corresponding to deviations from the regression between stations is significantly greater than that for the regression within stations. This suggests that some additional factor dependent on locality is operating, and the suggestion is confirmed by the fact that the two regressions differ significantly.

As there was a large variation in the proportions of yellow shells (ranging from 0 to 0·93), they were transformed to angles, and a weighted correlation coefficient between angle and mean body shade for the nineteen stations was calculated. The value obtained was 0·63, which with 17 degrees of freedom is significant at the 1 per cent level.

Consequently, there is some suggestion that the possession of a particular shell colour and pattern does affect the body shade of the individual. Snails with yellow and unbanded shells may have a tendency to lighter body colour. But this effect is insufficient to account for the strong connexion between a high percentage of yellow shells and a pale average body colour in the same colony, and conversely a low percentage of yellow shells and a dark average body colour.

Inspection of the collecting stations shows that the browner the backgrounds, the darker they are in shade. In the dense beechwoods, where there is little or no green undergrowth, the background is made up of red-brown leaves, dark brown twigs, cupules, and other litter, and exposures of dark soil. In the greener hedgerows and rough places, on the other hand, there is much pale leaf-litter, dry grass stems, and green grass, all of which form a background much lighter in shade than that of the beechwoods. It has been shown already (Cain and Sheppard, 1950) that the percentage of yellow shells in a colony is correlated with the greenness of the background. There is therefore also a correlation between the percentage of yellow shells and the lightness of the background.

Explanation of the correlation

There are four possible explanations of the correlation between light body-shade and high percentage of yellow shells in a colony:

(i) Individual animals may be able to change colour to harmonise with the background.

(ii) The food available may be different in different localities and may affect body shade.

(iii) The greener localities are less shaded than the dark ones, and the bodies of snails in greener areas may become bleached to some extent.

(iv) Body shade may be primarily genetically determined, and

subject to predatory selection of the same nature as that already shown to act on the shells.

The hypothesis of colour change in these animals is wholly unsupported by evidence; no such change has ever been reported, and contraction of pigment within the melanophores has not been observed. Moreover, the animals which were kept together under the same conditions showed no tendency to assume even approximately the same shade (table 4). Food is unlikely to affect melanin formation to the necessary degree. The animals kept together, although fed on the same diet, showed no disposition towards similarity. The same experiment gives no support for the hypothesis of bleaching. The container was shaded, and bleaching greatly reduced, if not wholly prevented. If lighter body colour in more exposed situations were due entirely to bleaching, then young animals taken from such situations and protected should become nearly as dark as the darkest adults found.

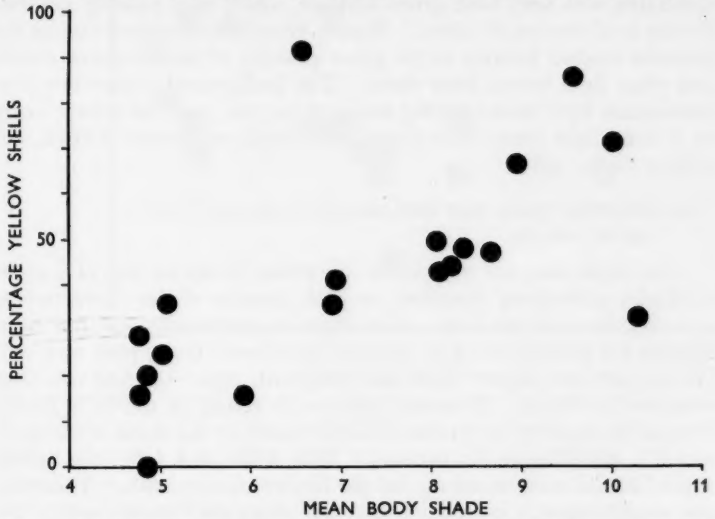

FIG. 1.—Correlation between mean body shade and percentage of yellow shells in each locality. ($P<0.01$). For discussion of the two divergent colonies see page 228.

If some of the paleness of juveniles were due to youth only, again they should darken with age. The experiment gives no indication of a general increase in darkness. Furthermore, juveniles collected in beech woods are generally very dark, some as dark as the darkest adults. There is no evidence of any extensive change of body shade with age.

It appears therefore that differences in body shade are primarily genetically determined. This hypothesis is supported by the relative stability of colour with age, and is further upheld by the significance of the bivariate regression of body colour on shell colour and banding, which shows that genes do affect body shade. The range of variation

within each colony indicates that, as might be expected, the control is multifactorial.

Both shell colour and body shade, therefore, are genetically determined. But the genetic effect of shell colour and banding on body shade is not sufficient to account for the correlation between mean body shade and the percentage of yellow shells within each colony. The factor dependent on locality which is producing this correlation can only be natural selection by predators, favouring light body shade on light backgrounds and dark shade on dark ones.

Fig. 1 shows the correlation between mean body shade and percentage of yellow shells in the localities examined. Two stations are somewhat divergent. The colony at Stretham has a high percentage of yellow shells but a dark mean body shade; that in Marley Wood, on the contrary, has a light mean shade for the percentage of yellow shells. At Stretham there was a long ditch with much dark soil, associated with very fresh green herbage, which may possibly account for this combination of values. Marley Wood is exceptional among the localities studied because of the great quantity of dead bracken fronds and other light brown litter there. The background is therefore predominantly light brown during much of the year, and the colony living on it has a light mean body shade and a high proportion of fawn, not yellow, shells.

The relationship between mean body shade and mean shell shade in the pink class

The shells that are not yellow are either brown or one of a series of shades collectively described as pink because of the characteristic ground-colour of the shell. This series is continuous and has been divided for scoring into four groups, light fawn, fawn, pink and red. (In our previous paper—Cain and Sheppard, 1950—the first two were combined as fawn.) If natural selection is acting on the body shade, it might be expected to act even more intensely on the shade of the shell, which is more frequently exposed. Within the pink series the lighter shades should be selected for on the lighter backgrounds. Therefore one would expect a correlation between mean shell shade (within the pink class) and mean body shade.

The same type of statistical analysis as used above was carried out, body shade being scored as before (0-20) and shell shade as 0-3 in order of increasing darkness, which is also the order of increasing redness. There is a highly significant correlation (fig. 2) between mean body shade and mean shell shade ($P<0{\cdot}001$), as expected. Moreover, there is no significant regression whatever of body shade on shell shade within colonies, so that again there is no single direct genetic connexion between the two. It has been shown (see Cain and Sheppard, 1950) that shell colour is not influenced by food. Fading is not in question here, since faded shells can be recognised as such, and included in the correct class. The shade of shell colour, therefore,

is also genetic and probably multifactorial. Moreover, because there is no correlation between body shade and shell shade in each individual, it is obvious that paleness cannot be due to fading in both.

Since there is a correlation between percentage of yellow shells within a colony and mean body shade, and between mean body shade and mean shade of pink, the possibility arises that yellow is incompletely recessive and that the paleness of the pinks in colonies with high percentages of yellows is due to a preponderance of heterozygous

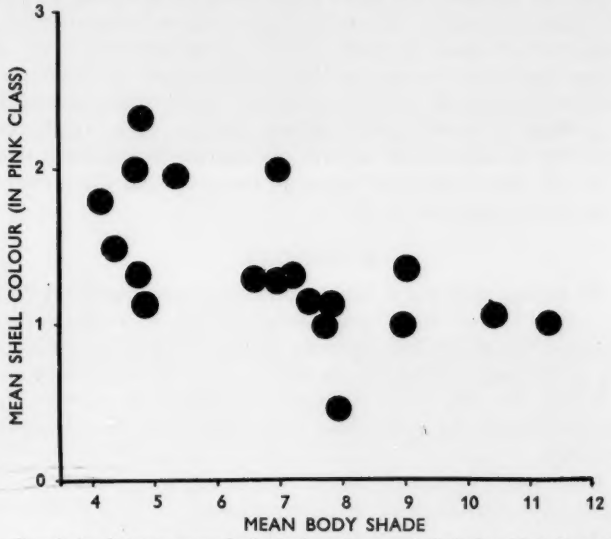

FIG. 2.—Correlation between mean body shade and mean shell shade within the pink class. ($P<0.001$.)

pinks. But this hypothesis does not fit the facts. Of the colonies already described (Cain and Sheppard, 1950, table 2), Putall Gate has a very low percentage of yellows (3·6) but a high proportion of the paler pinks, giving a pale mean shade of pink for the colony. Rockley, on the other hand, with a much higher percentage of yellows (27·5) has a higher proportion of the two darker classes. Other colonies show similar discrepancies.

The importance of natural selection in controlling variation

It appears, therefore, that the mean body shade, the mean shade of shell colour within the pink series, and the proportions of yellow and highly banded shells are all controlled by natural selection, not merely within a colony, but within different parts of a colony when there is variation in the background. Preliminary observation shows that the exact shade of brown also varies with the lightness of the background.

Sheppard (in press) has shown very striking variations in the proportions of varieties at Rockley over a distance of less than 100 yards. The correspondence of geographical variation in the colony and in the background is remarkable. Lamotte (1951) considers that breeding communities of *Cepaea* of radius less than 30 metres are panmictic. If so, then selection must be heavy to produce this correspondence.

In this species, then, we have major genes controlling colour and banding, and minor ones affecting shell and body shade; and both are controlled by selection. There are grounds for believing that the selection must be intense. It seems that whenever selection coefficients, even of apparently trivial characters, are adequately investigated, they are found to be surprisingly high (Mayr and Stresemann, 1950). Since conditions are never constant in nature, fluctuations in selection values are likely to be the rule, and will produce consequences identical with those ascribed to genetic drift (Sewall Wright, 1940, 1948) but not restricted by the size of the colony. It appears that natural selection is by far the most important agent in respect to all those characters that can be distinguished at all.

5. SUMMARY

1. In *Cepaea nemoralis* a high correlation was found between the percentage of yellow shells in a colony and the mean body shade in that colony, but hardly any between the shell colour and body shade in each individual. Animals with yellow unbanded shells tend to have paler bodies, but this effect is wholly insufficient to account for the general correlation between mean body shade and the percentage of yellow shells.

2. Body shade appears to be a genetic character, multifactorially controlled and unaffected by the environment.

3. The correlation of mean body shade with percentage of yellow shells is observed to be also a correlation with the general lightness or darkness of the background, and is the result of natural selection by visual predators.

4. This conclusion is confirmed by observation of the mean shade of shells in the pink class. The control of shell colour within this class is also multifactorial and highly correlated with the mean body shade in each colony, although not in each individual.

5. Natural selection, therefore, controls the frequency, not only of the major genes for colour and banding, but also of the numerous modifiers influencing the shade of the body, of the pink shells, and probably also of the brown shells.

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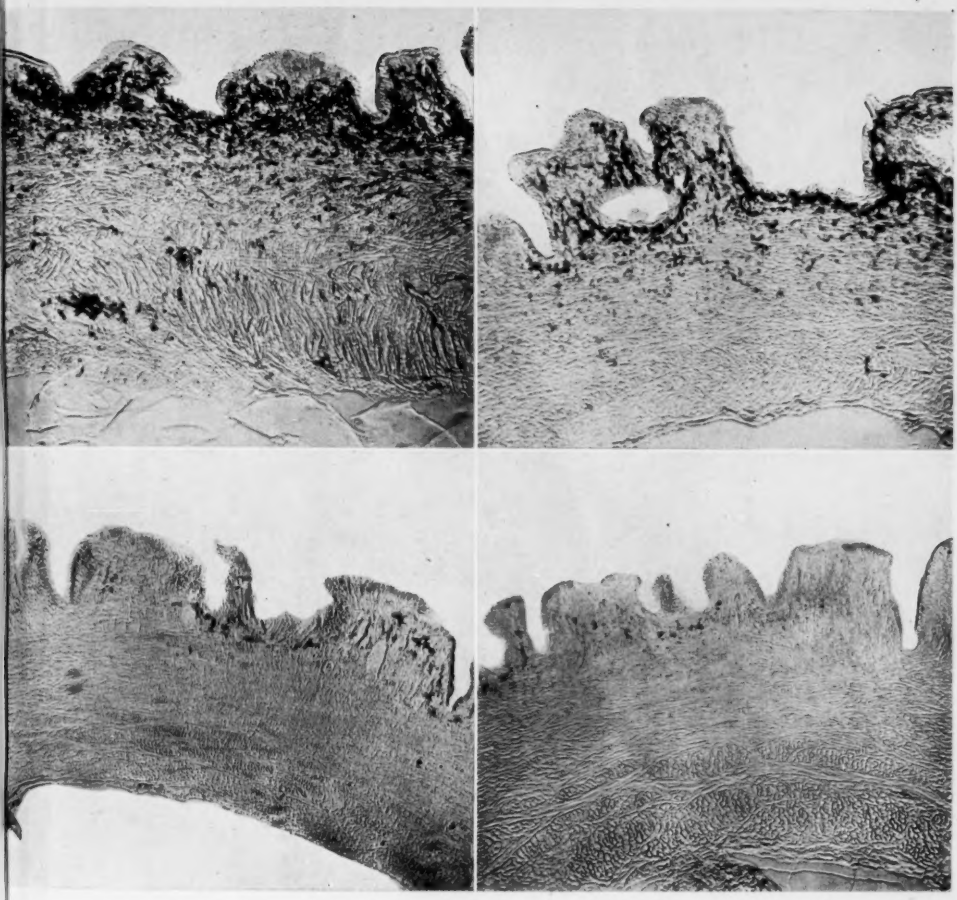

PLATE I.—Unstained transverse sections taken immediately behind the tentacles, showing degree of pigmentation. Body shade, previously determined, was 4, 7, 10 and 13 in figs. *a*, *b*, *c* and *d* respectively. All figures × 80.

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NATURAL SELECTION IN TWO COLONIES OF THE POLYMORPHIC LAND SNAIL *CEPAEA NEMORALIS*

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1. INTRODUCTION

It has been shown by Lang (1908 and 1912) and Fisher and Diver (1934) that the colour and banding patterns on the shell of the snail *Cepaea nemoralis* (L) are genetically determined. Diver (1940) not only found that colonies of the snails differ in the proportions of the varieties in them, but also that the ratio of the phenotypes may not be the same in all areas of a single colony. He maintained that this is the result of isolation by distance and sampling errors in reproduction, a process which is considered to be of great evolutionary importance by Wright (e.g. 1940). Cain and Sheppard (1950) and Sheppard (1951) on the contrary demonstrated that the various patterns have different selective values, and that their proportions, in any colony, are the result of natural selection; however, they did not preclude the possibility that small inbreeding communities might not diverge to some extent as the result of "genetic drift".

The present paper is an account of an investigation of a single colony of *C. nemoralis* and a comparison of this population with another, a mile away, which was first studied by Cain and Sheppard (1950).

2. METHODS

(i) *Collecting*

Samples of the shells of adult snails were taken from two colonies of *C. nemoralis* at Rockley and Rough Down near Marlborough, Wiltshire. To ensure that the collections were random, all the adult individuals that could be found were collected from several small areas in each population.

(ii) *Recording*

The colour and banding pattern of each shell was recorded using the method described by Cain and Sheppard (1950).

3. RESULTS

The results of collecting in the two colonies are given in tables 1 and 2. The samples from Rockley were taken from nine localities in

TABLE 1

Locality	Yellow				Pink				Brown		
	0(23)00	003(45)	00300	00000	(12345)	00345	00300	00000	(12345)	00300	00000
Rockley 1	—	—	5	1	—	—	1	4	1	1	8
Rockley 2	—	—	16	18	—	—	7	51	—	2	45
Rockley 3	—	—	10	15	—	—	4	20	—	—	21
Rockley 4	—	—	17	22	—	—	10	19	—	—	42
Rockley 5	1	—	48	51	—	—	11	25	—	2	63
Rockley 6	—	—	4	9	—	—	19	8	—	—	43
Rockley 7	—	1	7	7	—	—	3	4	—	1	11
Rockley 8	—	—	4	2	1	—	4	6	—	1	12
Rockley 9	—	—	3	1	—	1	6	2	—	3	9

TABLE 2

Locality	Yellow								Pink								Brown					
	(12345)	02345	023(45)	00345	00(345)	0(23)00	00300	00000	(12345)	02(345)	0(2345)	00345	003(45)	00(345)	00340	00300	00000	(12345)	02(345)	0(23)(45)	00(345)	00300	00000
Rough Down 1949	5	–	–	–	1	–	21	47	–	1	–	2	2	1	1	13	5	3	1	1	–	11	49
Rough Down 1950	5	–	1	–	–	1	9	29	1	–	–	–	–	–	–	2	1	–	–	–	–	–	18
Rough Down 1951	–	1	–	1	–	–	5	31	–	1	1	1	–	–	–	7	1	–	–	–	1	5	24
Total	6	1	1	1	1	1	35	107	1	2	1	3	2	1	1	22	7	3	1	1	1	16	91

a large area, all of which is inhabited by the snail (fig. 1). Three collections were obtained from Rough Down. The first was taken in 1949 (Cain and Sheppard, 1950), the second in 1950 and the third in 1951 at the same time as those from Rockley.

(i) *Description of the Rockley localities*

1. The area is covered by a thick plantation of beech trees with a carpet of beech cupules and small patches of exposed soil. There is also a quantity of sparse grass and herbs.

2. This locality is a continuation of the first but is on a steep slope and consequently much dryer. It has less green vegetation and there are large quantities of dry brown beech leaves on the ground as well as brown cupules.

3. The third station is a continuation of this wood. It becomes very narrow and ends as a single row of trees where it joins the sixth

locality. This narrow plantation has a thick carpet of grass with very few beech leaves or cupules. It is intermediate in type between the wood and green downland habitats.

4. The fourth locality is an open downland to the south-east of the second collecting station. For most of the year it has tussocks of short grass which have been flattened by the wind to give a smooth green carpet. However, since sheep no longer graze here the grass becomes quite long in late summer. The down is near a beech wood and consequently has a number of beech leaves between the tussocks. It is therefore intermediate in type between beech wood and downland.

5. The fifth area is a continuation of the preceding locality. It is an open downland habitat with exactly the same vegetation as the fourth station but with no beech leaves on it as it is further from the wood.

6. The snail population in the sixth locality is living under a very overgrown beech hedge which is about twenty feet high and ten feet wide. Both the grass field to the north of the hedge and the cart track to the south are unsuitable for *C. nemoralis* and one not permanently inhabited by it. The ground under the hedge is devoid of vegetation over considerable areas, but there are patches of grass wherever there are few branches near the ground or where there are gaps between the bushes.

7. To the south of the beech hedge and east of the large beech wood (area 9) there is a continuous stretch of downland which surrounds a triangular plantation. For most of the year this downland is covered by tussocks of medium length grass which has been flattened by the wind to give a smooth green carpet.

8. In area 7, about 60 yards to the south of the hedge, near its northern end, there is a small triangular beech plantation. This has a carpet of beech leaves very similar to area 2.

9. A hundred yards west of the triangular copse there is a large beech wood with a carpet of litter very like that found in the preceding locality.

FIG. 1.—Sketch map showing the collecting localities at Rockley. Broken lines mark their boundaries. The woods are all Beech woods.

(ii) *Description of the Rough Down locality*

The collections from Rough Down were taken from a steep downside of short grazed turf. This colony is on the same escarpment as part of the Rockley population but is about a mile to the south-east of it. The two colonies are separated from one another by a road and a number of ploughed fields.

It will be seen from tables 1 and 2 that the breeding communities in the beech wood areas have a much lower percentage of yellow shells than those living in the downland stations. This agrees with the results obtained by Cain and Sheppard (1950). Moreover, the intermediate types of habitat (area 3, which is a beech wood with a thick carpet of grass, and area 4, which is downland with some beech leaves on it) are intermediate with respect to the percentage of yellow shells in the populations.

The Rough Down colony also has a high proportion of yellow shells and agrees with the two Rockley downside communities.

4. DISCUSSION

Cain and Sheppard (1950) showed that there is a relationship between the ratio of the different colour and banding varieties in a population and the background on which it lives. They demonstrated that colonies living on a green background have a high proportion of yellow shells and the more discontinuous the background the higher the percentage of banded shells. Moreover, they suggested that snails with bands one and two absent are more unbanded than banded in appearance and should be classified as unbanded for ecological though not, of course, for genetical purposes. Lamotte (1950) has also shown that the different banded varieties can have different selective values but unlike Cain and Sheppard (1950) he believes that this selection is not usually of any importance (1951).

The percentage of each of the three main colour phases (brown, pink and yellow) from the nine Rockley stations and Rough Down have been plotted on a triangular diagram (fig. 2). It will be seen that the samples from the beech wood habitats (areas 1, 2, 6, 8 and 9) fall into one group on the diagram, whereas those from the two Rockley downland areas and the Rough Down colony form another group. The two populations from the intermediate type of habitat (areas 3 and 4) fall between these two groups. Moreover, the proportion of yellow snails in the downland areas is significantly higher than in the beech wood habitats.

The correspondence between the proportion of the genetically determined colour varieties and the background on which the population lives can only be adequately explained by natural selection. Moreover, the results confirm the conclusions of Cain and Sheppard (1950) which were based on a comparison of samples from many different colonies. Furthermore, the divergence in the gene frequencies in the different collecting stations, within a single colony, as a result of natural selec-

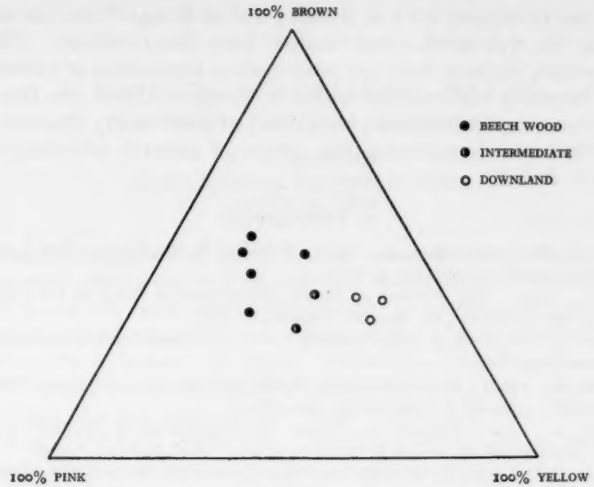

FIG. 2.—The triangular diagram shows the percentage of yellow, pink and brown shells in samples from the Rockley and Rough Down populations. Note that localities classified together because of their ecological similarity (downland, beech wood or intermediate) tend to group together.

tion, is in direct agreement with Diver's (1940) view that there is considerable isolation between the different parts of one colony as a result of distance. However, it contradicts his conclusion that the observed differences in the composition of breeding communities are the result of " genetic drift ". Lamotte (1951) has estimated the effectiveness of isolation by distance in *C. nemoralis* and has arrived at a value for the size of the panmictic breeding unit, which would account for the amount of divergence found at Rockley if selection is intense.

It is probable that the intermediate values of the gene frequencies in areas 3 and 4 at Rockley are, in part, the result of incomplete isolation between the beech wood and downland habitats, as well as of the pressure of natural selection in the area itself. However, it is true to say that breeding communities in quite small parts of a continuously inhabited area can diverge from one another as the result of natural selection.

5. SUMMARY

1. The distribution of colour and banding varieties of the snail *Cepaea nemoralis* was investigated in two colonies at Rockley and Rough Down, a mile apart.

2. The Rough Down population lives in only one type of habitat. The population at Rockley extends over both downland and beech wood, and over two ecologically intermediate areas.

3. Three varieties, pink, brown and yellow, are found in all parts of the Rockley colony as well as at Rough Down. Their proportions

in the two downland areas at Rockley and at Rough Down are similar, whereas the five beech wood stations have fewer yellows. The two intermediate habitats have an intermediate proportion of yellows.

4. Breeding communities within a colony of *Cepaea* can thus show differences in gene frequency as a result of isolation by distance. But the differences demonstrate the action of natural selection, not of " genetic drift ".

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A NOTE ON NON-RANDOM MATING IN THE MOTH *PANAXIA DOMINULA* (L)

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In animals, examples of genes having marked morphological and physiological effects are well-known, but the control of behaviour by specific genes has been very little studied. Consequently, few genes are known which regulate the mating behaviour particularly with regard to the "choice" of mates. Moreover, most reports of non-random mating between individuals differing by a single major gene, suggest that like genotypes usually tend to mate together. Therefore it seems worthwhile recording the following observations on the effects of a single gene (not excluding other genes very closely linked with it) on mating behaviour.

In the moth, *Panaxia dominula* (L.), a gene showing no dominance is found at an appreciable frequency in one colony in Berkshire (Fisher and Ford, 1947). The heterozygote is recognisable as var. *medionigra* and the rarer homozygote as var. *bimacula*. It has been suggested (Kettlewell, 1942) that mating between the three genotypes is not random. Because this observation was not based on strictly numerical data an experiment was designed to test the hypothesis (Sheppard, 1951). Although a tendency for unlike genotypes to pair was observed, the data were not extensive enough for useful conclusions to be based on them. Consequently, the work was repeated and extended in 1951. The results of this new study together with the data previously reported (Sheppard, 1951) are given in table 1.

There is a very strong tendency ($P<0.001$) for unlike genotypes to pair, and the result is not due to a different sex drive in the males of the three varieties. This can be verified, for *medionigra* and *bimacula*, from lines 7 and 8 in the table in which the only differences between the cages concern the females. A change from a *bimacula* to a *medionigra* female resulted in the *bimacula* males mating more often (table 1, column 8), whereas before the *medionigra* males paired more frequently. Preliminary observations on the mating behaviour suggest that the disassortative pairing is controlled by the female. On several occasions she has been observed to reject actively a male of her own genotype, and has then at once accepted a male of a different form. The observations suggest that the courtship between like genotypes is longer than between unlike forms as the male will usually succeed in pairing with the female in the end, providing that a moth of another genotype is not present. The shorter the courtship the less chance there is of

the other male in the cage interrupting it and mating with the female first. If there is only one male, then with a long courtship, it is more likely to be attracted by another female before copulation takes place than if the courtship is short.

TABLE 1

	Genotypes of the three moths in the cage	Result of first mating		Totals
		Like genotypes pairing	Unlike genotypes pairing	
1	*dominula* ♂ *dominula* ♀ *medionigra* ♀	8	20	28
2	*medionigra* ♂ *dominula* ♀ *medionigra* ♀	12	14	26
3	*dominula* ♂ *medionigra* ♂ *medionigra* ♀	13	14	27
4	*dominula* ♂ *medionigra* ♂ *dominula* ♀	11	22	33
5	*medionigra* ♂ *medionigra* ♀ *bimacula* ♀	2	0	2
6	*bimacula* ♂ *medionigra* ♀ *bimacula* ♀	0	1	1
7	*medionigra* ♂ *bimacula* ♂ *bimacula* ♀	3	15	18
8	*medionigra* ♂ *bimacula* ♂ *medionigra* ♀	2	10	12
9	*dominula* ♂ *dominula* ♀ *bimacula* ♀	2	1	3
	Totals	53	97	150

In a number of insects the phenotype has been disguised with cellulose paint. All three insects in the cage were marked with paint so that the phenotypes were not treated differently. Not enough results have been obtained for any definite conclusions to be formed, but the data suggest that the mating behaviour is not influenced by visual stimuli. It is likely that scent is important as females use it to " assemble " the males, and, in butterflies at least, males are known which employ " scent organs " to induce the female to mate (Eltringham, 1933).

It is not known whether this disassortative mating takes place in the wild but it is probable that it does to some extent. Even if the

female of any genotype is equally attractive to all the males, the control exercised by the female will have some effect as she often " assembles " a large number of males before she mates. This will result in competition between the males, and those which have a short courtship time will have a better chance of mating. If this tendency to non-random mating occurs in the wild, the number of heterozygotes will be increased above the value expected in a random mating system. Moreover, because the males, but not the females, mate more than once, the *medionigra* males will have a better chance of mating than the wild type males if the gene-frequency be low. This will put them at a slight selective advantage. If this was the only character controlled by the gene, which it is not, stable polymorphism would result. It would be interesting to know if a tendency for unlike genotypes to mate is as common in nature as the reverse situation.

The apparently widespread tendency for genes to have manifold effects is of considerable importance from the evolutionary point of view, because the more characters a gene controls the more likely it is that any change in the environment will alter its selective value. Wright (1932) has suggested that fluctuations in gene frequency are favourable to rapid evolution and has maintained that for this reason " genetic drift " must be particularly important. However, as Fisher and Ford (1947, 1950) have pointed out, directly it is established that selection is also markedly effective in causing gene-frequency fluctuations then " drift " loses its special importance in evolutionary theory.

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THE FREQUENCY OF THE A B O BLOOD GROUPS IN DUBLIN

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A RECENT analysis of the records of the National Blood Transfusion Association of Ireland has yielded the following numbers of people of groups *O*, *A*, *B* and *AB* living in the Dublin area. As far as is known, there has been no selecting of people of a particular group.

TABLE 1

The total data

Group	Number	Percentage
O	5050	*53·79207*
A	3079	*32·79719*
B	1019	*10·85428*
AB	240	*2·55646*
Total . .	9388	*100·00000*

TABLE 2

Numbers of men and women of groups O and A

	O	A	Total	$\frac{A}{A+O}$%
Men . .	3279	2035	**5314**	38·295
Women .	1771	1044	**2815**	37·087
Total .	5050	3079	**8129**	...

$\chi^2 = 1{\cdot}14129$ for 1 degree of freedom
P $= 0{\cdot}30$ (or $0{\cdot}15$ if direction is taken into account)

While these relatively small numbers do not reveal any significant sex difference, it should be noted that the percentage difference of the $\frac{A}{A+O}$ ratio ($+1{\cdot}208$) is in the same direction and of the same order of magnitude as that discovered in an analysis of English data (Fisher and Roberts, 1943). In this latter study the large numbers involved revealed the difference to be significant.

Table 3 sets out the present data, together with independent data previously published for this area.

Thus the data may be considered homogeneous. From the totals for the *O*, *A* and *B* groups the gene frequencies and the expected number in group *AB* can be calculated, assuming a random mating system, by the method proposed by R. A. Fisher (Race and Sanger, 1950).

Frequency of gene $O = 0{\cdot}7278$
Frequency of gene $A = 0{\cdot}1982$
Frequency of gene $B = 0{\cdot}0740$

1·0000

TABLE 3

Source	O	A	B	AB	Totals
Boyd and Boyd * .	220	124	48	7	399
Sachs (1940) . .	1305	789	280	61	2,435
Hooper (1947) . .	2394	1557	569	123	4,643
Present data . .	5050	3079	1019	240	9,388
Totals . .	8969	5549	1916	431	16,865
Percentages . .	53·18114	32·90246	11·36081	2·55559	100·00000

χ^2 for 9 degrees of freedom = 11·26985
P = 0·25

* Cited by Weiner (1943).

Expected number in group $AB = 496{\cdot}4337$ with variance of 668·5923.

When the observed and the expected numbers of people in group *AB* are compared ; $\chi^2 = 6{\cdot}40385$ for 1 degree of freedom. P = 0·01. Thus there is a significant deficiency in group *AB*. It is worth enquiring specifically whether the four independent sets of data are homogeneous for deficiency in this group.

If *o*, *a* and *b* are the frequencies of genes *O*, *A* and *B* calculated from the grand totals for groups *O*, *A* and *B* (these frequencies are given above), and for any one set of data the observed total number in the 3 groups *O*, *A* and *B* is *n*, and the expected total number in all four groups is N, then :—

$$n = \mathrm{N}(1-2ab)$$

$$\mathrm{N} = \frac{n}{1-2ab}$$

Expected number of $AB = \mathrm{N}-n = \frac{2ab}{1-2ab}n$

This can be shown to have a variance of $\frac{2abn}{(1-2ab)(a+o)(b+o)}$

In this way the expected number of people of group *AB* can be

calculated for each set of data and then compared with the observed number by a χ^2 test. The agreement between the sets of data can be estimated by calculating the heterogeneity χ^2 as summarised in table 4.

TABLE 4

Source	AB observed	AB expected	AB observed − AB expected	Variance	χ^2
Boyd and Boyd	7	*11·8414*	− 4·8414	15·9479	1·46975
Sachs	61	*71·7131*	−10·7131	96·5826	1·18832
Hooper	123	*136·5389*	−13·5389	183·8893	0·99681
Present data	240	*276·3403*	−36·3403	372·1724	3·54839
Summed χ^2 (4 degrees of freedom)					7·20327
Total data	431	*496·4337*	−65·4337	668·5922	6·40385
Heterogeneity χ^2 (3 degrees of freedom)					0·79942

P = 0·85

Thus all the sets of data agree in showing a deficiency in group *AB*. It is emphasised that the calculation of the expected numbers in group *AB* implies a random mating system and no differential survival and it is probable that one of these assumptions is unjustified. The first possibility that presents itself is that the assumption of a random mating system is invalidated by the barriers that tend to prevent intermarrying of Roman-Catholics and Non-Roman-Catholics. It is a reasonable guess that the gene frequencies among the Non-Roman-Catholics (many of whom are descendants of English settlers) are roughly half-way between the average frequencies for England and Dublin. The gene frequencies for the Non-Roman-Catholics can then be calculated knowing that there are 498,233 Roman-Catholics and 52,492 Non-Roman-Catholics in the area (Census of Population, 1946), and assuming our total data to constitute a representative sample. The results of these calculations are set out in table 5.

TABLE 5

	o	*a*	*b*	Expected number in group AB
England (Dobson and Ikin, 1946)	0·6831	0·2569	0·0600	...
Dublin (present data)	0·7278	0·1982	0·0740	...
Dublin Non-Roman-Catholic	0·7055	0·2275	0·0670	49·0047
Dublin Roman-Catholic	0·7302	0·1951	0·0747	446·6659
				495·6706

Thus there is a slight tendency for the *A*'s and *B*'s to be separated by this division. The expected total number of *AB*'s is calculated assuming random mating within each group but no inter-marrying. This total (495·6706) is to be compared with the expected number assuming a completely random mating system (496·4337) and the observed number (431). Even allowing for considerable inaccuracies in the suggested gene frequencies in these two groups, it is clear that the religious barriers will not account for a deficiency in group *AB* of the magnitude of that observed. Further elucidation of the problem is not possible from the present data.

It is a pleasure to thank Dr E. Hackett for placing the Blood Group records of the National Blood Transfusion Association at my disposal and to Mr S. W. Glover for assistance in classifying these records.

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PRELIMINARY DATA ON CROSSING OVER BETWEEN *H-2* AND *Fu*, *Ki* AND *T* IN THE MOUSE *

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1. INTRODUCTION

PREVIOUS work (Gorer, Lyman and Snell, 1948 ; Snell and Higgins, 1951 ; Snell, 1951) has demonstrated the existence on chromosome 9 of the mouse of a locus, histocompatibility-2 (*H*—2), important in determining susceptibility and resistance to tumour transplants. Five alleles have been identified. These are *H*—2, characteristic of strain A ; *H*—2^d, characteristic of strains DBA/2 and BALB/*c* ; *H*—2^b, characteristic of strains C57BL/6 and C57BL/10 ; *H*—2^p, characteristic of strain P ; and *H*—2^k, characteristic of a non-inbred kinky line called K8 and probably also of strain CBA.

The locus, histocompatibility-2, is closely linked with fused tail (*Fu*), kinky tail (*Ki*) and brachy or short tail (*T*). Because classification of animals as genetically susceptible or resistant on the basis of tumour inoculation is subject to occasional error, results so far published have failed to demonstrate conclusively the occurrence of crossing over between *H*—2 and the other three loci. This paper is concerned with the demonstration of such crossing over.

The data come from several different types of crosses. None of these was designed primarily to provide linkage information but all produced such information as a useful byproduct.

2. LINKAGE WITH *T*

The gene *T* was introduced into strain A by 4 or 5 successive back-cross matings. It brought with it into the cross a histocompatibility-2 allele distinct from *H*—2, and probably identical with *H*—2^b (unpublished data). The resulting stock was crossed again to mice of strain A. This cross was therefore

$$H\text{—}2^bT/H\text{—}2+ \times H\text{—}2+/H\text{—}2+$$

Brachy offspring could be either *H*—2^bT/*H*—2+ (non-crossovers) or *H*—2*T*/*H*—2+ (crossovers).

* This investigation has been assisted by a grant from the National Cancer Institute and by a Grant-in-aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council.

Non-brachy mice were discarded, and the brachy mice were mated to strain C57BL/6. This mating was

$H-2^bT/H-2+ \times H-2^b+/H-2^b+$ for non-crossovers,
$H-2T/H-2+ \times H-2^b+/H-2^b+$ for crossovers.

Non-brachy mice were discarded and brachy mice inoculated subcutaneously with strain A tumour 15091*a*. Non-crossovers should throw, except for new crossovers, all *T* resistant (−) offspring; crossovers should throw all *T* susceptible (+) offspring. The inoculation of 5 young was set as a satisfactory test, but owing to poor breeding by some of the brachy mice, particularly by brachy females, this number was not always attained.

The results are summarised in table 1. Results of tests where only 1, 2 or 3 *T* young were inoculated are included in this table, but are not used in the totals or in any subsequent tables. For animals tested by 4 or 5 or more inoculated offspring, the crossover values were 4·1 per cent. for heterozygous males (total of 73 mice) and 12·5 per cent. for heterozygous females (total of 8 mice). The combined figure is 4·9 per cent.

TABLE 1

Number of brachy (T) *mice and number of presumed brachy crossovers from the mating* H−2+/H−2^{b}T×H−2+/H−2+, *grouped according to the number of brachy* (T) *offspring raised and inoculated to test each animal. Only mice tested by 4 or more inoculated young are used in calculating crossover per cent.*

	Number of *T* offspring inoculated						Crossover per cent.
	1	2	3	4	5 or more	Total (last 2 columns)	
Mice by heterozygous males—							
Number of mice . . .	2	6	10	15	58	73	
Number with all positive offspring	0	1	1	1	2	3	*4·1*
Mice by heterozygous females—							
Number of mice . . .	0	1	3	2	6	8	
Number with all positive offspring	0	1	1	0	1	1	*12·5*

Combined per cent. with all positive offspring (crossover per cent.) = 4·9±2·4

The one crossover in the group tested by 4 inoculated *T* offspring was additionally tested by the fact that he had been used prior to testing, in matings to A females, to sire some mice who were themselves tested. All of six such offspring proved to be genetically $H-2T/H-2+$. He was therefore unquestionably a crossover. It remains to consider the adequacy of the test applied to the mice from whom 5 or more *T* young were raised and inoculated.

Table 1 shows that there were 3 probable crossovers tested by the inoculation of 5 or more offspring. Actually 1 of these was tested

by the inoculation of 12 young, all of which were positive, 1 by the inoculation of 9 young, all of which were positive, and 1 by the inoculation of 5 young, all of which were positive. There would seem to be no question that the first two were crossovers. Data pertinent to the question as to whether the third may have been diagnosed falsely as a crossover are given in tables 2 and 3.

From table 2 it will be seen that *T* males diagnosed as non-crossovers gave 32 offspring that grew the tumour and 256 that failed to grow it, while for *T* non-crossover females the figures were 20 and

TABLE 2

Outcome of tumour inoculation of brachy (T) *offspring by brachy non-crossover males and females from cross* $H-2+/H-2^{b}T \times H-2^{b}+/H-2^{b}+$

Sex of parent	Positive	Negative	Per cent. positive
Male . . .	32	256	*11·1*
Female . . .	20	99	*16·8*
Total . .	52	355	*14·6*

99. This is 11·1 per cent. and 16·8 per cent. positive respectively, with a combined value of 14·6 per cent. Some of the positive mice could have been, and presumably were, crossovers. However, the combined value is significantly higher ($P = \cdot 04$) than the combined crossover per cent. shown in table 1. Presumably, then, some of the positive mice in table 2 must be otherwise explained.

In view of the well-known fact that some tumours will grow in a percentage of animals of certain foreign and hence presumably resistant strains, the obvious explanation is that some of the genetically resistant mice succumbed. Our past experience would have led us to expect such false positives to be rare with tumour 15091*a* when the genotype of the inoculated animals was $H-2^{b}/H-2^{b}$. However, some of the stocks used in this cross were obviously in rather poor physical condition, due to unknown causes, and this may have decreased resistance to the tumour. In any case we regard the figure 4·9 per cent. from table 1 as the more reliable measure of crossing over between *H*—2 and *T*.

Table 3 shows the frequency distribution of brachy males and females from which 5 or more young were inoculated, tabulated according to the per cent. positive offspring. It will be seen that 2 out of 61 non-crossovers gave 50-60 per cent. positive offspring, but that none gave more than 60 per cent. It seems unlikely therefore that any non-crossovers would give 5 out of 5 or 100 per cent. positive offspring. The one mouse previously mentioned, which was diagnosed as a crossover on the basis of 5 positive offspring, is not likely to have been incorrectly classified.

Error from false negatives is ruled out by the fact that all inoculated mice were genetically F_1 hybrids between strains A and C57BL/6

TABLE 3

Frequency distribution of brachy males and females from which 5 or more young were inoculated, tabulated according to per cent. positive offspring

	Non-crossovers					Crossovers
Per cent. positive .	0	12-20	27-40	50-60	61-99	100
Sex of parent—						
Male . .	28	10	6	1	0	3
Female . .	7	2	6	1	0	0

except for the chromosome segment under test. The crossover value of $4{\cdot}9 \pm 2{\cdot}4$ per cent. should therefore be reliable.

3. LINKAGE WITH *Fu*

The data came from two different sources.

A chromosome segment tagged by the gene *Fu* was introduced into strain A by repeated backcrosses. This segment was derived from the CA strain, genetically *CaCaFufuWw*, and carried with it a histocompatibility-2 allele distinct from *H*—2 (Snell and Higgins, 1951) but not otherwise identified. The mice were thus $H-2^{x}Fu/H-2+$. A number of them, from several different backcross generations, were used in crosses set up to identify the *H*—2 allele present in certain inbred strains.

One mouse, male ACA80, in the third backcross generation, mated to strains C57BL·10, RIII and ST, gave 25 normal tailed young and 24 fused tail young which all succumbed to strain A tumour 15091*a*. Since strains C57BL/10, RIII and ST all lack allele *H*—2 (Snell and Higgins, 1951 and unpublished data), male ACA80 must have had the genotype $H-2Fu/H-2+$. This genotype could have been derived by mutation from $H-2^{x}$ to *H*—2. Since there are at least 5 (and probably more) alleles at the histocompatibility-2 locus, there is a degree of improbability in the assumption that mutation would give the particular allele born by the homologous chromosome. The acquisition of *H*—2 by crossing over seems more probable.

The total number of tested mice from the backcross of *Fu* to strain A was 18. Only male ACA80 proved to be a crossover. The indicated crossover per cent. is 5·6 (table 4), but the probable error is of course high.

Additional information comes from another group of crosses. These had the form

$$(M \times F^{h}) \times N \text{ or } (M \times F^{H}) \times N$$

where M and N are any two inbred strains, F^{h} is a strain carrying

the gene *Fu* linked with an unidentified histocompatibility-2 allele which however was not *H*—2, and F^H is a strain carrying the gene

TABLE 4

Number of mice and number of genetically tested crossovers from mating H—2+/H—2*Fu×H—2+/H—2+

Number of mice	Number of crossovers	Per cent. crossovers
18	1	5·6

Fu linked with *H*—2. Offspring of the double cross were inoculated with a tumour native to the strain in the M position.

The results are summarised in table 5.

TABLE 5

Crossover data from crosses involving fused (Fu). *Animals were classified as positive* (+) *only if they succumbed to the tumour*

Cross	Tumour	Results			
		++	+−	*Fu*+	*Fu*−
(A×F^A)×C57BL/6, C57BL/10, C57BR/cd, C57L, MA, RIII *	*15091a*	31	11 (3 §)	1 ‡	34
(BALB/c×F^A)×A, AK, C3H, C57BL/10, C57BR/cd, C57L, LINE 11, P, RIII, ST	*S621*	75 (1 †)	13	4 (3 ‡)	80
(C57BL/6×F^H)×A, AK, BALB/c, DBA/2, P, RIII	*C1498*	55	15 (1 §)	2 (1 ‡)	58
Total	...	161	39 (4 §)	7 (5 ‡)	172
(C57BL/6×F^H)×ST . .	*C1498*	7	4	5 (1 ‡)	15

* In this group, data from some crosses were excluded on the ground that the inbred strain used in the final cross shows partial susceptibility to 15091a. For example, about 30 per cent. of DBA/2 mice succumb to 15091a, probably because of the virulence of the tumour plus a relationship between the alleles *H-2* and *H-2*^{d} (Snell, 1951). Since all *Fu* mice from this cross would receive the allele *H-2*^{d} from the DBA/2 parent, some of the *Fu* animals might be expected to succumb even though possessing a genotype ordinarily classified as resistant.

† Because S621 and C1498 are slightly less virulent tumours than 15091a, and because good data for choosing between the more and less satisfactory crosses were lacking, all crosses were included in these two cases. However, data for the cross involving C57BL/6 and ST appear aberrant and are given separately.

‡ These mice succumbed to the tumour, but survived longer than other susceptible mice. They probably should be classified as genetically resistant. For detailed data on length of survival of comparable cases see table 7 of Gorer, Lyman and Snell (1948). The numbers outside the parentheses are the totals, including the presumably resistant mice.

§ These mice had normal tails but were proved by genetic tests to carry the fused gene.

It will be seen that in general the non-fused mice succumbed to the tumour while the fused mice survived. The exceptions (+ − and *Fu*+ mice) may be crossovers, but we need first to exclude other possibilities. The gene *Fu* is known to be subject to the rather frequent occurrence of normal overlaps (Reed, 1937). There is every reason to suppose that many of the normal tailed resistant (+ −) mice belong in this category. Some were proved to belong here by genetic tests. These are indicated in the + − column of table 5 by the numbers in parentheses. The occurrence of these overlaps renders the normal tailed (++ and + −) mice unsuitable for use in estimating crossover per cent. We shall therefore confine our consideration to the fused mice.

Some of the fused survivors may possibly have been genetically susceptible and hence crossovers. However, we have good evidence from several sources that 15091*a* usually kills any mice with the allele *H*−2. (See for example, the results of inoculating offspring of male ACA80 referred to above.) The evidence is not so clear in the case of the other tumours, but in any case the frequency with which " susceptible " mice survive is low and the resulting error small.

More important is the occurrence of false positives. We have already pointed out that these occur in discussing the linkage of *H*−2 and *T*. A clue as to which animals fall in this category may be obtained from the records as to the length of survival of, and progress of tumour growth in, the *Fu*+ mice. Normal tailed susceptible mice inoculated with 15091*a* usually die in 3 to 5 weeks and very rarely live to 8 weeks. The one mouse included in table 5, first cross, which was *Fu* and which succumbed to tumour 15091*a*, lived 10 weeks and showed partial tumour regression at 4 to 6 weeks before the final spurt of tumour growth which killed it. Presumably this mouse was genetically resistant. Other such mice are indicated in parentheses in the *Fu*+ column.

The *Fu*+ mice which succumbed after the normal interval are probably crossovers. Possible exceptions are the 4 *Fu*+ mice from the cross $(C57BL/6 \times F^{H}) \times ST$ which succumbed promptly to tumour C1498. These are separated from the total because the high number of positives in this one cross suggests some unusual condition. Omitting both the long survivors and the mice from this one cross, there are 2 probable crossovers out of 174 mice. The indicated crossover value is 1·2 per cent. However, the sources of error are obviously such that this value should be regarded as possibly subject to future emendation. Its principal utility lies in the indication that the accurate but limited data in table 4 may give too high a figure.

4. LINKAGE WITH *Ki*

Data pertinent to the linkage of *H*−2 and *Ki* were derived from crosses of the type

$$(A \times K) \times N$$

where A is strain A, N is any other inbred strain (all strains used lacked *H*—2) and where K is a strain carrying *Ki* associated with a histocompatibility-2 allele other than *H*—2. Mice from the double cross were inoculated with strain A tumour 15091*a*. Results are summarised in table 6. The indicated crossover value, including

TABLE 6

Crossover data from crosses involving kink (Ki). All mice were inoculated with A strain tumour 15091a. Mice from crosses to strains DBA/2 and ST are excluded because these strains show partial susceptibility to this tumour. Mice were classified as positive (+) only if they succumbed to the tumour.

Cross	++	+−	*Ki*+	*Ki*−
(A×K)×AK, CBA, C57BL/6, C57BL/10, P	47	4*	1	48

* Mice tested genetically, not normal overlaps.

all mice, is 5 per cent., and including only *Ki* mice, 2 per cent. Since none of the presumed crossovers were proved susceptible or resistant by a breeding test, some question must remain as to the correctness of the classification. The occurrence of crossing over between *H*—2 and *Ki* should be confirmed by further tests before the separability of the *H*—2 and *Ki* genes is regarded as proved.

5. DISCUSSION

Dunn and Caspari (1945) have shown that *Fu*, *Ki* and *T* lie in a chromosome segment not exceeding 8 units in length. The occurrence of overlaps in fused heterozygotes and certain other difficulties inherent in the nature of the material leave some uncertainty as to the details of the arrangement. The best available estimates of crossover per cent. are :—

Fu and *T*, 4·3 per cent. in heterozygous males.

Ki and *T*, 4·3 per cent. average for both males and females, with the rate being higher in females than in males. Another cross gave 4·8 per cent. in males.

Fu and *Ki*, 2 per cent. The poor viability of *FuKi* mice makes this cross particularly difficult.

The order of the four identified loci on chromosome 9 is uncertain, but there is indication that *H*—2, *Fu* and *Ki* are bunched at one end of the linkage group in an interval of perhaps 2 units, while *T* is separated from the nearest of the other three by perhaps 4 units.

6. SUMMARY

1. The locus histocompatibility-2 (*H*—2), which is important in determining susceptibility and resistance to tumour transplants, lies on chromosome 9 in close association with the genes *Fu*, *Ki* and *T*.

2. Crossing over between *H*—2 and *T* was 4·9± 2·4 per cent.

3. Crossing over between *H*—2 and *Fu* was 5·6 per cent. in one cross and an estimated 1·2 per cent. in another. The first figure is based on small numbers and the second is derived from a cross where there is a considerable element of uncertainty in the detection of crossovers. Further data are needed.

4. There is evidence indicating but not finally proving that crossing over occurs between *H*—2 and *Ki*.

5. The locus of *H*—2 is distinct from the loci of *T*, *Fu* and probably *Ki*, but its order relative to these loci is not yet determined.

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LOCATION OF "REELER" IN LINKAGE GROUP III OF THE MOUSE

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"REELER" (*rl*) is a recessive gene of the house mouse which impairs locomotion through a disturbance of balance. It has been tested for linkage with a few markers, with which it was found to segregate independently (Falconer, 1951). The loci of macrocytic anæmia (*W*) and of luxate (*lx*), in linkage group III were, however, not included in these tests, and subsequent work has shown reeler to be linked with these two loci, the order being *W*—*lx*—*rl*, and the recombination 16 per cent. with *lx* and 29 per cent. with *W*. This linkage is interesting in that it adds another 16 units to this already long linkage group, but its practical value in linkage studies is not likely to be great on account of the poor breeding performance of reeler homozygotes. On the other hand the linkage may have some value as an aid in the maintenance of reeler stocks.

The data which give evidence of the linkage come from four types of mating, of which the parental genotypes are shown in table 1. The viable allele of macrocytic anæmia (W^v) was used in the tests. Some of the matings segregated all three loci, but in the presentation and analysis of the data the two tests, *rl* with W^v and *rl* with *lx*, are treated separately. Reeler segregated always from an intercross; the other loci segregated either from an intercross or a backcross, and in each case the coupling and repulsion phases were represented, though not always equally. The distribution of the progeny of each type of mating among the different phenotypic classes is given in table 1.

The analysis of the data was made by means of Fisher's scoring system (Fisher, 1946), and the scores (S) and amounts of information (I) for each type of mating are set out in table 2. The columns headed 1 are based on scoring coefficients calculated for the assumption of free recombination ($p = 0{\cdot}5$) and undisturbed single-factor segregations. The highly significant values of χ^2 disprove the hypothesis of independent segregation beyond doubt, and the analyses indicate approximate recombination fractions of 35 per cent. with W^v and 25 per cent. with *lx*.

The more precise estimation of the recombination fractions is complicated by the faulty single-factor segregations of both W^v and *lx*. There is a deficiency of W^vW^v phenotypes which may be attributed

to prenatal mortality, and a deficiency also of *lxlx* phenotypes which may be attributed, in part at least, to linkage in coupling with W^v.

TABLE 1

Observed segregation of rl *with* W^v *and with* lx

Type of mating	Genotype of Female	Genotype of Male	+ W^vW^v	+ W^v+	+ ++	*rl* W^vW^v	*rl* W^v+	*rl* ++	Total
A	$\frac{W^v+}{+rl}$	$\frac{W^v+}{+rl}$	6	22	11	2	4	9	54
B	$\frac{W^vrl}{++}$	$\frac{W^vrl}{++}$	13	85	33	10	12	2	155
C	$\frac{+rl}{++}$	$\frac{W^v+}{+rl}$	...	13	7	...	2	8	30
D	$\frac{+rl}{++}$	$\frac{W^vrl}{++}$	...	33	51	...	13	11	108
			lxlx	*lx*+	+	*lxlx*	*lx*+	+	
A	$\frac{lx+}{+rl}$	$\frac{lx+}{+rl}$	10	41	19	2	2	25	99
B	$\frac{lx\ rl}{++}$	$\frac{lx\ rl}{++}$	7	22	61	7	1	5	103
C	$\frac{lx+}{+rl}$	$\frac{++}{+rl}$	...	14	29	...	0	6	49
D	$\frac{lx\ rl}{++}$	$\frac{+rl}{++}$	...	9	32	...	3	8	52

In addition, *lx* shows normal overlapping of the heterozygote class. In order to make allowance for these disturbed segregations the scoring coefficients were recalculated on the basis of the observed frequencies of the W^vW^v, *lxlx*, and *lx*+ phenotypic classes. Thus the expected frequencies for the single-factor segregations were as follows : for intercrosses,

Phenotype .	W^vW^v	W^v+	++	*lxlx*	*lx*+	+	
						lx+	++
Expectation .	0·15	0·56	0·29	0·13	0·32	0·26	0·29

and for luxate in backcrosses,

Phenotype .	*lx*+	+	
		lx+	++
Expectation .	0·26	0·24	0·50

The recombination values assumed in the re-scoring were those indicated by the first analysis, 35 per cent. with W^v and 25 per cent. with *lx*. The scores and information obtained with the revised coefficients are given in the columns headed 2 in table 2. The

estimates of the recombination fractions are now substantially lower than those obtained from the first analysis, 30 per cent. with W^v and 16 per cent. with *lx*, the reduction being due to the removal

TABLE 2

Analysis of the data in table 1

Type of mating	W^v						*lx*					
	1 ($p = 0{\cdot}50$)		2 ($p = 0{\cdot}35$)		3 ($p = 0{\cdot}30$)		1 ($p = 0{\cdot}50$)		2 ($p = 0{\cdot}25$)		3 ($p = 0{\cdot}15$)	
	S	I	S	I	S	I	S	I	S	I	S	I
A	21	*144*	0	*161*	−10	*188*	80	*264*	28	*178*	11	*263*
B	59	*413*	34	*410*	21	*463*	80	*275*	19	*309*	−16	*514*
C	16	*40*	9	*43*	7	*46*	2	*65*	4	*34*	4	*51*
D	16	*144*	−5	*155*	−13	*165*	5	*69*	−3	*22*	−5	*22*
Total . .	**112**	*741*	**38**	*769*	**5**	*862*	**167**	*673*	**48**	*543*	**−6**	*850*
χ^2 . .	*17*		*1·9*		*0·025*		*41*		*4·2*		*0·04*	
Estimated recombination	35%		30%		29%		25%		16%		16%	
Standard error	...		...		3·4%		...		...		3·4%	

S = Score. I = Amount of information.

of the disturbing effects of the aberrant single-factor segregations. A third analysis based on the revised recombination fractions was made, and the results are given in the columns headed 3 in table 2. The values of χ^2 testing deviation from the assumed recombination fractions are now very small, and the final estimates of recombination with their standard errors are

$$W^v\text{—}rl : p = 29 \pm 3{\cdot}4 \text{ per cent.}$$
$$lx \text{ —}rl : p = 16 \pm 3{\cdot}4 \text{ per cent.}$$

These values accord well with the recombination of $17{\cdot}7 \pm 1{\cdot}2$ per cent. observed for W^v with *lx* (Carter, 1951), and the order of the three loci is proved to be W^v—*lx*—*rl*.

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EPISTATIC RATIOS INVOLVING TWO PHENOTYPES

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1. INTRODUCTION

MANY characters require the normal (wild-type) reactions of many nonallelic genes in serial order for the production of a normal phenotype; these normal reactions may be engendered by dominant or recessive genes. Those alleles of the normal genes that inhibit, prevent, or obscure any reaction in such a series are epistatic and result in the absence of the normal character. In cases where several pairs of normal and epistatic alleles segregate, the characteristic polyhybrid segregation ratios may be considerably modified. The calculation of these complex F_2 or testcross epistatic ratios by means of the dichotomous, algebraic, or checkerboard methods is onerous and usually somewhat protracted. The following methods for determining epistatic ratios involving two phenotypes are short, accurate, and relatively simple; and they may be employed practically in breeding programmes and for Chi Square tests.

2. CALCULATING F_2 PHENOTYPIC RATIOS INVOLVING EPISTASIS

These F_2 ratios may be calculated as follows:

(1) Determine the F_2 combinations that permit the expression of the character, *i.e.* the hypostatic combinations.

(2) Determine the total F_2 genotypic combinations involved in the cross from 4^n, where n = the number of segregating pairs.

(3) Determine the hypostatic phenotypic coefficient of the combination or combinations permitting the expression of the character from 3^d, where d = the number of allelic pairs that may be represented by one or two dominant genes.

(4) Determine the phenotypic coefficient of the epistatic class or classes by subtracting the hypostatic phenotypic coefficient from the total F_2 genotypic coefficient.

Therefore, the F_2 ratio is equal to

$$3^d : 4^n - 3^d.$$

The following examples illustrate the use of this method:

Illustrative Problem: In a species of plant the production of coloured flowers requires the simultaneous presence of the normal dominant allele of three genes, namely, *A*, *B*, and *C*, and of the normal recessive allelles of two genes homozygously, namely *ii* and *jj*. If the recessive

alleles of any or all of the first three named genes are homozygous, *i.e. aa*, *bb*, or *cc*, or one dominant allele of either of the last two named genes is present, *i.e. I* or *J*, no pigment will be produced, and the flowers will be white.

What F_2 phenotypic ratios would be expected from each of the following crosses?

(*a*) *AAbb* × *aaBB*
(*b*) *AABBCC* × *aabbcc*
(*c*) *AAii* × *aaII*
(*d*) *IIJJ* × *iijj*
(*e*) *AABBii* × *aabbII*
(*f*) *AABBCCii* × *aabbccII*

Solution:

Colour produced by	Total F_2 Genotypic Combinations (4^n)	Phenotypic Coefficient of those coloured (3^d)	Phenotypic Coefficient of those not coloured ($4^n - 3^d$)	F_2 Phenotypic Ratio coloured : white
(a) *A-B-*	$4^2 = 16$	$3^2 = 9$	$16 - 9 = 7$	9 : 7
(b) *A-B-C-*	$4^3 = 64$	$3^3 = 27$	$64 - 27 = 37$	27 : 37
(c) *A-ii*	$4^2 = 16$	$3^1 = 3$	$16 - 3 = 13$	3 : 13
(d) *iijj*	$4^2 = 16$	$3^0 = 1$	$16 - 1 = 15$	1 : 15
(e) *A-B-ii*	$4^3 = 64$	$3^2 = 9$	$64 - 9 = 55$	9 : 55
(f) *A-B-C-ii*	$4^4 = 256$	$3^3 = 27$	$256 - 27 = 229$	27 : 229

3. CALCULATING TESTCROSS PHENOTYPIC RATIOS INVOLVING EPISTASIS

These testcross ratios may be calculated as follows:

(1) Determine the testcross combination that permits the expression of the character, *i.e.* the hypostatic combination or genotype.

(2) Determine the total testcross genotypic combinations involved in the cross from 2^n, where n = the number of segregating pairs.

(3) The hypostatic phenotypic coefficient of the combination permitting the expression of the character is always 1.

(4) Determine the phenotypic coefficient of the epistatic classes by subtracting the hypostatic phenotypic coefficient from the total testcross coefficient.

Therefore, the testcross ratio is equal to

$$1 : 2^n - 1.$$

The following examples illustrate the use of the testcross method:

Illustrative Problem:—Using the same genes as those used in the previous problem, what testcross ratios would be expected from testcrossing the F_1's from each of the following crosses?

(*a*) *AAbb* × *aaBB*
(*b*) *AABBCC* × *aabbcc*
(*c*) *AAii* × *aaII*
(*d*) *IIJJ* × *iijj*
(*e*) *AABBii* × *aabbII*
(*f*) *AABBCCii* × *aabbccII*

Solution:

Colour produced by	Total Testcross Genotypic Combinations (2^n)	Phenotypic Coefficient of those coloured (1)	Phenotypic Coefficient of those not coloured ($2^n - 1$)	Testcross Phenotypic Ratio coloured : white
(a) *AaBb*	$2^2 = 4$	1	$4 - 1 = 3$	1 : 3
(b) *AaBbCc*	$2^3 = 8$	1	$8 - 1 = 7$	1 : 7
(c) *Aaii*	$2^2 = 4$	1	$4 - 1 = 3$	1 : 3
(d) *iijj*	$2^2 = 4$	1	$4 - 1 = 3$	1 : 3
(e) *AaBbii*	$2^3 = 8$	1	$8 - 1 = 7$	1 : 7
(f) *AaBbCcii*	$2^4 = 16$	1	$16 - 1 = 15$	1 : 15

4. SUMMARY

Short methods are presented for determining F_2 and testcross epistatic ratios involving two phenotypic classes. These methods may be used conveniently for a great variety of combinations embracing epistasis.

THE GENETIC SITUATION CONCERNING FERMENTATION IN YEASTS

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THE knowledge of the genes in yeasts which are responsible for fermentation of different sugars is now so advanced that we are able to form an idea of the general principles according to which fermentative ability is inherited.

In yeasts as in several other fungi tetrad analyses are possible, since in many cases 4-spored asci are formed and the spores are easily isolated and grown separately. Hence the segregations from artificially produced hybrids between species with different fermentative abilities may be studied. In most yeast species, however, linear tetrads similar to those occurring in *Neurospora* and several other fungi are not formed, as the ascus is very often ellipsoidal. This is, for example, the case in many *Saccharomyces* types, while in *Schizosaccharomyces Pombe* Leupold (1950) had no difficulty in studying ordered tetrads since the asci in this species are cylindrical and the spores arranged in a single row.

The most important fact that has been established is that the genes in question are inherited in a strictly mendelian manner and that apparently all genes for fermentation are dominant. Another fact is that several (polymeric) genes for the fermentation of a given sugar may be present in one yeast (Winge and Roberts, 1948, 1950).

GALACTOSE FERMENTATION

A simple mendelian segregation is observed when the progeny of the hybrid *Saccharomyces cerevisiae* × *S. Chevalieri* is analysed. The former species is able to ferment galactose rapidly (i.e. within 1-2 days), while the latter is a slow fermenter, which was originally described as unable to ferment galactose. It ferments, however, galactose after an adaptation period of 4-6 days. The hybrid segregates out from every ascus two spores giving rise to rapidly fermenting cultures and two giving rise to slowly fermenting cultures. Exactly the same result is obtained when the progeny of the hybrid *S. Italicus* × *S. Chevalieri* is analysed; here it is *S. Italicus* which is the rapid fermenter. In our own experiments we have not made crosses with species which were entirely unable to ferment galactose, but Lindegren (1949 and earlier) mentions a segregated type which could not adapt to galactose-fermentation, and the same type has later been mentioned by Kilkenny and Hinshelwood (1951). A hybrid between this type and *S. cerevisiae* gave also a strictly mendelian ratio of 2 fermenters : 2 non-fermenters. As we know, some

species have been described, as for example *S. Bayanus*, which are unable to ferment galactose.

It is still uncertain whether three alleles exist, G for rapid fermentation, g_s for slow fermentation and g for no fermentation, or whether they are non-allelic. A hybrid Ggg_sg_s would of course segregate out 2 rapid : 2 slow fermenters—i.e. it would give the same segregation as a Gg_s type in which G and g_s were alleles.

It has been claimed by Lindegren and his co-workers that our g_s-gene for slow fermentation of galactose does not actually exist, but that a non-fermenter gene, g, mutates to G during the fermentation experiment. Our g_s-type should therefore represent a mixture of G- and g-cells. It has, however, been made sufficiently clear that this is not the case; Spiegelman, Sussman and Pinska (1950), Spiegelman (1950) and Kilkenny and Hinshelwood (1951) were able to verify our statement that it is not a question of mutation but of long-term adaptation, as we had originally termed the phenomen (Winge and Roberts, 1948). Spiegelman and his collaborators, however, claim, that only a few of the cells of a culture can adapt to galactose fermentation. In their opinion some cytoplasmic units are active during adaptation. As the problem of adaptation has no direct relation to inheritance, I shall not go into details regarding this interesting subject.

MALTOSE FERMENTATION

It has been shown (Winge and Roberts, 1948, 1950*a*) that in *Saccharomyces cerevisiae* (strain " Yeast Foam " from U.S.A.) not less than three genes for maltose fermentation are present. We have called them M_1, M_2 and M_3. A hybrid between this yeast and *S. Chevalieri*, which is unable to ferment maltose, will yield a surplus of fermenters from single spore cultures. We have shown that the fermenters and non-fermenters are segregated out in ratios which are in accordance with the assumption that the M-genes are situated at a sufficient distance from the centromeres of the respective chromosomes so that there is 50% crossing-over between the genes and their centromere. The three M-genes are not linked to each other. Therefore a triple hybrid M_1m_1 M_2m_2 M_3m_3 will segregate fermenters and non-fermenters at a ratio of 7M : 1m and the tetrad analyses will show 52·8% asci segregating as 4M : 0m, 44·4% segregating as 3M : 1m, and only 2·8% asci segregating as 2M : 2m. A doubly heterozygotic hybrid obtained through back crossing will segregate out fermenters and non-fermenters in the proportion of 3 : 1, and the tetrad analyses will show 16·7% asci segregating as 4M : 0m, 66·7% segregating as 3M : 1m, and 16·7% segregating as 2M : 2m. Our findings are in full agreement with these ratios. Our triply heterozygotic hybrid yielded the following ratios:

Segregation . .	4M : 0m	3M : 1m	2M : 2m
Observed . .	30	16	1
Theoretical . .	24·8	20·9	1·3

Our doubly heterozygotic hybrid yielded the following ratios:

Segregation . .	4M : 0m	3M : 1m	2M : 2m
Observed . .	37	154	36
Theoretical . .	37·9	151·4	37·9

To prove fully the correctness of this theory, it was necessary to isolate each of the three M-genes by backcrossing spores from the triple hybrid to the recessive *S. Chevalieri*. We succeeded in this and have produced a number of yeast types containing M_1 alone, others with M_2 alone, and several yeast types which only possess M_3. It is easily established whether M_1, M_2 or M_3 is present in a yeast, since a cross between two yeast types with different M's will give a doubly heterozygotic hybrid, while the crossing of two yeasts with identical M's will yield a non-segregating hybrid, the offspring of which will all be fermenters.

It is quite interesting that a new M-gene, designated M_4, was obtained through irradiating a recessive non-fermenter (Winge and Roberts, 1950). It arose as a heterozygotic, segregating 2M : 2m in each ascus, as expected, and a priori we supposed that this M would turn out to be either M_1 or M_2 or M_3. By crossing the new M-type to M_1, M_2 and M_3 we found, however, that in all cases a double heterozygote was obtained. Hence the new M-gene was not identical with any of the three old M-genes, and was accordingly called M_4. Later on the same M_4-gene arose spontaneously from the same non-fermenting culture.

As the experiments distinctly show that the origin of M_4 did not involve the mutation of an inhibitor gene masking the effect of an already existing M_4-gene, we favour the idea that a hypothetical " progene ", ineffective but closely related chemically to M_4, may already have been present in the non-fermenting yeast type. This ineffective gene may have been altered through a single mutational step to a maltose fermenting gene, and thus may have occurred spontaneously or through irradiation.

The fact that the existence of not less than four genes for maltose fermentation has been demonstrated compels one to question whether they are biochemically identical. It might seem easy to compare the effect of the four M-genes on a series of sugars, but if certain other fermenter genes are also present in the yeast types, it is necessary to eliminate them before the effect of the M-genes can be tested. As an example we had in our M_2-types a gene, R_1, for raffinose fermentation; this R-gene is responsible not only for the splitting and partial fermentation of raffinose but also for the fermentation of sucrose. Consequently it is impossible to state whether M_2 is able to ferment sucrose (in addition to maltose) as long as R_1 is present. It is necessary to produce a yeast type containing M_2 but lacking R_1 in order to establish whether or not M_2 is responsible for sucrose fermentation.

At present only M_1 and M_2 have been investigated in this way, and

the results have shown (Winge and Roberts, 1952) that the two genes are biochemically different; M_1 is sucrose as well as maltose fermenting while M_2 is only maltose fermenting. In other words M_1 is a gene for the production of α-glucosidase while M_2 is a gene for the production of maltase. The problem of an M-gene being able to ferment both sugars has been previously discussed by Gilliland (1949), who regarded the gene which we have called M_1 as a gene responsible for the production of α-glucosidase.

Of course not all maltose fermenting yeast species contain as many as three or four M-genes. *Saccharonyces Italicus*, for example, is a maltose fermenter, and Gilliland (*l.c.*) demonstrated that it possessed only one M-gene pair. He crossed *S. Italicus* to the non-fermenting species *S. Chevalieri* and found that the hybrid regularly segregated out 2 fermenters : 2 non-fermenters from each of 20 asci. Later on Dr Roberts and I (1950*b*) identified this M-gene of *S. Italicus* with our M_1 gene in *S. cerevisiae*.

It would be interesting to ascertain whether M_3 and M_4 are biochemically different from M_1 and M_2. Lindegren, who was the first to observe segregation with respect to maltose fermentation, maintains (1949, page 26-5) that " asci do not follow any regular behaviour ", and he claims to have frequently observed non-mendelian ratios, as, e.g., 1M : 3m. According to Lindegren and his colleagues (e.g. Mundkur, 1949*a*, *b*) deviating ratios are explained by " gene-conversion ". We have never observed non-mendelian ratios or any sign of " gene-conversion ". We have, however, pointed out several sources of error when yeast hybrids are analysed; these may involve overlapping of generations, 8-nucleate asci, and mutations. We have not been able to identify any of our four M-genes with the gene for maltose fermentation, MA, described by Hestrin and Lindegren (1951). As Lindegren probably has more than one M-gene in his material even though he does not admit this possibility, his MA gene may actually comprise more than one single gene.

RAFFINOSE AND SUCROSE FERMENTATION

Three genes responsible for the fermentation of raffinose have also been demonstrated in a single yeast type. Gilliland's hybrid *Saccharomyces Italicus* × *S. Chevalieri* was found to be triply heterozygotic, segregating out according to the ratio of 7 raffinose fermenters : 1 non-fermenter (1949). By analysing 20 asci of the hybrid, he found exactly 70 fermenters and 10 non-fermenters—namely 12 asci yielding 4R : 0R, 6 asci yielding 3R : 1r, and 2 asci yielding 2R : 2r. According to theoretical expectation, 10·6, 8·9 and 0·6 respectively of the three types of asci should have occurred among the 20 analysed. It could not be a doubly heterozygotic segregation, and therefore there could hardly be any doubt that three gene pairs were involved.

A closer examination of this hybrid was undertaken (Winge and Roberts, 1952), and we succeeded in separating the three R-genes in

different yeast types just as we had previously done with the three maltose fermenting genes in *S. cerevisiae*.

Yeasts containing an R-gene will split raffinose as well as sucrose. They produce a β-h-fructosidase, which splits off fructose from the raffinose molecule. The sucrose is fermented, leaving melibiose untouched. Hence, if only R is present in the yeast, only one third of the raffinose is fermented.

The three genes for raffinose fermentation were called R_1, R_2 and R_3. If two yeast types containing unlike R-genes are crossed, e.g. R_1 and R_2, a double heterozygote, $R_1r_1\ R_2r_2$, will be produced, which will give a two-gene segregation. If two yeast types with identical R-genes are crossed the hybrid will of course be homozygotic as to the R-gene in question, and consequently all offspring will be raffinose-fermenting.

CLOSE LINKAGE BETWEEN M_1 AND R_1

Gilliland's original analyses of the hybrid *Saccharomyces Italicus* × *S. Chevalieri* disclosed a peculiar dependence between maltose and raffinose fermentation in the offspring of this hybrid, as all of his 10 non-fermenters of raffinose were able to ferment maltose.

According to our present knowledge the following formula can be ascribed to this hybrid:

S. Italicus	×	*S. Chevalieri*
$M_1M_1\ GG\ r_1r_1\ r_2r_2\ r_3r_3$		$m_1m_1\ g_sg_s\ R_1R_1\ R_2R_2\ R_3R_3$

Hybrid

$M_1m_1\ Gg_s\ R_1r_1\ R_2r_2\ R_3r_3$

The hybrid segregates out from every ascus 2 maltose-fermenters to 2 non-fermenters of maltose, and 2 rapid galactose-fermenters to 2 slow galactose-fermenters. As to raffinose fermentation, as we have already seen, there are different possibilities, some asci segregating out as 4 : 0, others as 3 : 1, and again others as 2 : 2. When, however, all non-fermenters of raffinose are found to be maltose fermenters, there must be some state of dependence between the M_1-gene and one or several genes for raffinose fermentation.

Our own experiments aimed at an explanation of this phenomenon.

Tetrad analyses of a hybrid of the above named formula (Winge and Roberts, 1952) were in full accordance with Gilliland's results; we found 2 : 2 segregations as to maltose fermentation in all of the 37 asci analysed and 15 raffinose-negative single spore cultures, all of which were able to ferment maltose.

The explanation was found to be that the M_1-m_1 and R_1-r_1 gene pairs were closely linked. Through backcrossing the hybrid offspring to the raffinase-recessive type of the same formula as *S. Italicus* we produced the type $\overbrace{M_1m_1R_1r_1}\ r_2r_2\ r_3r_3$, and by analysing spore tetrads from this we obtained according to expectation a most peculiar segre-

gation. From 62 asci analysed we found in every case the segregation $2M_1r_1 : 2m_1R_1$—i.e., two single spore cultures fermenting maltose but not raffinose, and two cultures fermenting raffinose but not maltose. Furthermore we found one case of crossing-over—namely, an ascus segregating out as $1\ M_1r_1 : 1m_1R_1 : 1\ M_1R_1 : 1\ m_1r_1$.

By crossing the two cross-over types, which after diploidisation had the formula $M_1M_1\ R_1R_1$ and $m_1m_1\ r_1r_1$ respectively, we obtained a new hybrid, $\overbrace{M_1\underbrace{m_1\ R_1}r_1}$ with linkage in the opposite direction, since the two dominant genes were now linked. 11 tetrads from 4-spored asci were analysed and 10 of these yielded the segregation $2\ M_1R_1 : 2\ m_1r_1$—i.e., two single spore cultures fermenting maltose as well as raffinose, and two cultures which were unable to ferment either of the two sugars. In one of the 11 asci crossing-over had occurred, and hence the segregation was $1\ M_1R_1 : 1\ m_1r_1 : 1\ M_1r_1 : 1\ m_1R_1$.

The linkage between M_1 and R_1 thus has been verified by analysing the phenomenon in the coupling phase as well as in the repulsion phase. The distance between the two genes has been calculated to be about 1·3 centimorgans.

As already stated, M_1 as well as the R-genes cause sucrose fermentation, but there is a distinct difference between them as to the fermentation interval—i.e., the time that elapses between the start of the fermentation test and the first appearance of CO_2 in a Winge fermentometer. While an R-type is able to ferment sucrose within 5 hours, an M_1-type requires one to several days.

Summary.—As will be evident from these analytical results, all genes so far investigated are inherited in a strictly mendelian way. All fermenter genes, one for galactose fermentation, four for maltose fermentation, and three for raffinose and sucrose fermentation, show dominance. While the maltose fermenting genes M_1 and M_2 have been shown to have different biochemical effects, it remains to be determined whether M_3 and M_4 are different from M_1 and M_2 and whether or not the three R-genes are biochemically identical.

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REVIEWS

THE GENETICS OF MICRO-ORGANISMS. By D. G. Catcheside. Pitman & Sons, 1951. Pp. vii+223. 21s.

The rapidly advancing study of microbial genetics is of special importance not only in its theoretical implications but also in its practical consequences. In the first place, the very short generation times involved make microbes ideal material for the experimental investigation of mutation, selection and evolution. The existence of readily accessible haploid stages permits the immediate recognition of genes which might otherwise be masked by dominant partners. A wide variety of mutants are available, including nutritional, enzymatic and serological characters, as well as phage and drug resistance. Further, in some strains at any rate, genetic recombination can be demonstrated and tentative chromosome maps constructed. In the second place, the great progress made in recent years in the chemotherapeutic treatment of disease has given some prominence to the phenomenon of drug resistance. One of the urgent tasks of microbial genetics is to shed further light on drug-resistant mutations with a view to improving the clinical efficiency of antibiotics. We may also expect to develop in due course adequate explanations of microbial evolution which will increase our understanding of the way in which epidemics are caused by the sudden spread of new virulent pathogenic organisms through a community.

The genetics of micro-organisms by Prof. Catcheside is " based on a course of lectures devised for bio-chemists specialising in the microbial aspects of their subject. It attempts to provide an introduction to the general and special aspects of the genetics of micro-organisms." This book can scarcely be regarded as elementary; it presupposes in the reader a fair knowledge of genetics, cytology, biochemistry and microbiology. For those who have the entrée to these subjects a wealth of detailed and painstakingly compiled factual information on a considerable range of fascinating topics is presented and discussed. There are chapters on " Genetic analysis in *Neurospora* and other fungi ", " Mutation and gene action ", " Adaptation and mutation " and " Sexual reproductive systems ", followed by four chapters devoted to yeasts, protozoa, bacteria and viruses, respectively.

In a quickly expanding and developing subject like microbial genetics it is inevitable that any text-book, however well written, will be to some extent out of date in a relatively short time. (It should be noted that the preface to the book under review was written in February 1949.) These consequences will be minimised if the author adopts an historical approach. If he describes when, how and by whom certain researches were undertaken, and how the experimental results were interpreted in relation to the ideas and knowledge of the time, then the treatment may continue to have value long after many of the theories outlined have in fact been proved false. Unfortunately, Catcheside's book does not fulfil these conditions, though it could easily have done so with only slight changes in the method of exposition. Names of individuals and the dates of their work are few and far

between in the text, and it is very rare for both a name and a date to appear together. This is a result of the inconvenient method of documentation adopted. All references are made to a *numbered* entry in the bibliography given at the end of the chapter. Thus in the description of Sonneborn's fundamental work on cytoplasmic inheritance in *Paramecium*, apart from the date (1937) one is given merely a cryptic " 158 ". Sonneborn's name is discovered only by hunting for " 158 " in the bibliography. It would have been much better to have given a textual reference in the form: (Sonneborn, 1937). References of the latter kind coupled with a purely alphabetical bibliography would not only be much easier to use but would make the whole treatment far more readable. Incidentally, as the bibliography contains no page references to the sections of the book where authors' names appear, it is extremely difficult to find where the work of any particular author is discussed.

In a book of this sort one does not, of course, expect any very detailed mathematical discussions, though formulae given should be clear and unambiguous. This desideratum is not always fulfilled. On p. 28 there is a reference to the use of a product formula for the estimation of a recombination fraction allowing for differential viability. Those familiar with such methods will immediately recognise what is intended, but the student would find it far less confusing to be presented with a straightforward formula for the recombination fraction x in terms of the observations, say a, b, c and d, such as

$$x = (ab/cd)^{\frac{1}{2}} / \{1 + ab/cd)^{\frac{1}{2}}\}.$$

Moreover, even if statistical methods are considered beyond the scope of the book, references to the relevant literature should not be omitted. For example, in the chapter on " Genetic analysis in *Neurospora*, etc. ", although there is a detailed discussion of tetrad analysis, no mention is made of the important paper by Mather and Beale (*J. Genetics*, *1942*) on the calculation and precision of linkage values from tetrad analysis.

A minor misprint occurs three lines from the bottom of p. 118 where the right hand side of the differential equation should presumably contain an additional factor $1/P$, otherwise the equation will not be satisfied by the solution given in the last line on the page.

Rather more serious confusion arises in some of the mathematical formulae on pp. 159-161, where ambiguities are caused by faulty typography. Thus in line 8 on p. 160, $-\log_e C_o/C$ should read $-\log_e(C_o/C)$. On the other hand, in the following line, $m \log_e 2/N$ should read $m(\log_e 2)/N$. For similar reasons, all the other formulae are liable to misinterpretation by the student seeking to use them, as without prior knowledge it is impossible to guess where the brackets should be inserted.

Throughout the book no very adequate distinction is made between recombination and map distance. Indeed on p. 25, lines 24-28, it is implied that distance *measured as recombination percentage* is additive, which is certainly not true in general though it may be sufficiently accurate for short segments. Similarly, in lines 8-11 on p. 185, 14% recombination is equated to 14 crossover units, whereas on the assumption of no interference Haldane's formula gives a distance of rather more than 16 cM. Moreover, the 14 is incorporated in the chromosome maps of *Escherichia coli* on p. 186, where the values taken from Lederberg are certainly map distances and not recombinaton fractions. Finally, the estimates of the recombination fractions between the

loci *t* and *l*, and between *b* and *m*, which Catcheside has made on the basis of Lederberg's data, are invalid. As Lederberg himself rightly pointed out, the data only permit the estimation of the recombination fraction between *th* and *m* or *b*. We cannot, for example, use the data to estimate the extent of recombination between *b* and *m* without knowing the order of the loci *th*, *b* and *m*, which is uncertain. Similarly with *t* and *l*.

In spite of the above criticisms of detail, this book is certainly one of the first attempts to provide a broadly based and comprehensive text-book on microbial genetics, and it will no doubt do much to encourage further interest and research.

NORMAN T. J. BAILEY

GENETICS IN THE 20TH CENTURY. Essays Edited for the Genetics Society of America by L. C. Dunn, The Macmillan Company, New York. 1951.

This volume contains twenty-six essays presented at the " Golden Jubilee of Genetics " meeting of the Genetics Society of America. Most aspects of genetics are covered and a short review can do no justice to such a pleasantly varied collection of essays, none of which is irrelevant. I shall therefore pick a few points which have struck me as unexpected or more important.

In the first place, an impression which I hope to be wrong: in some of the theoretical parts of papers dealing with fundamentals one senses a somewhat defensive attitude; this is reflected in a categorical re-statement of concepts—e.g. unequivocal separation of hereditary and non-hereditary changes; genetic " control " of development; randomness of mutation, etc. —the usefulness of which we know to be the greater the more constantly aware we are of their limited validity and the more eager we are to replace them as soon as a higher synthesis is possible. Mendel's independent assortment was broadened to include linkage; his elements have been replaced by the functions of definite chromosome regions; particulate heredity has been harmonised with Darwinism; the gene-character gap is still there but at least we begin to see clearly the nature of the problem. Are we to believe that concepts as those mentioned above (and even more, that of " self-reproducing " particles) are eternal? If this stiffening attitude were the result of the bitter attacks now raging against genetics, both from the obscurantist biological right and the political extreme left, this would be indeed a victory for the attackers.

Fortunately in other theoretical essays, or theoretical parts of essays, there is no trace of this psychological weakness. Mather, writing on " Progress and Prospect of Biometrical Genetics ", definitely uses the past as a stepping-stone for the future. So do Sonneborn and Ephrussi in their outstanding essays on cell heredity and differentiation. So do Darlington and Lederberg in those parts of their essays where they unify infection and heredity. There is no trace of defensive attitude in the numerous essays which deal with particular fields of experimental genetics or with the application of genetics to agriculture, medicine, and, more generally, human affairs. This is natural because who grapples daily with practical problems of experimental design and analysis in genetics cannot fail to be fascinated by the tremendous predictive value of genetical theory though he realises its limitations. Even Huxley, in his beautiful closing essay, raises well above the stiffening attitude, in which he has recently indulged, when he tries to visualise on the basis of genetical knowledge the novel evolution made

possible in man by the emergence of mind and by the pooling of experience through tradition.

A second point which has struck me as requiring searching analysis, is the extensive and loose use now prevalent of the concept of " self-reproducing " particles or structures. A salutary criticism is to be found at the end of Sonneborn's essay. We all agree that there are cell structures ranging from chromosomes and their parts to viruses and transforming principles, which the cell is usually unable to synthesize *de novo*, and that certain changes in such structures lead to synthesis of replicas of the changed type. If we use the term " self-reproduction " in this descriptive sense, and remember the simple analogy with the " self-reproduction " of glycogen in the presence of a trace of it, of the appropriate enzymes and of glucose-phosphate, there is no harm. However, there is an animistic tendency to attribute to genes, viruses and other such particle properties well beyond these. When we speak of the gene as reproducing *itself*, and *controlling* a metabolic process we tend to think of it as a *wee one*; but immediately afterwards we see it as a macromolecule. This makes for unclear thinking; the sooner we stop using the term " self-reproduction " the better; " genetic continuity " is safer, and the emphasis should shift on to the action of all these structures as specific primers and the part they play in the synthetic processes of the cell.

A third point is the small amount of attention which is at present paid to the problems of spatial organisation of biochemical processes in the cell. Apart from a passing remark by Sturtevant on the promise of position effects, a reference by Darlington to Peters' ideas and one by Sonneborn on the possible assembly-line systems of enzymes on the microsomes there is barely any mention of this problem. Yet, as the reviewer has been stressing for some time, biochemistry is reaching a dead end if it does not find means to bring in the space variable, and genetics has to offer, with crossing-over, a tool of structural analysis the extraordinary resolving power of which is unlikely to be equalled for a long time.

It is impossible to mention here all the twenty-six essays, let alone to do them justice. Some are of historical character and they make most instructive reading. Some are reviews of well delimited fields—such as one on immunological genetics by Irwin, one on chemical genetics by Beadle, one each on cytochemistry by Mirsky and by Caspersson and Schultz, one on bacterial genetics by Lederberg, one on the genetics of cancer by Little, one on population genetics by Dobzhansky, an excellent one on hybrid corn by Mangelsdorf, one on genetics and plant pathology by Walker. Others, like the short stimulating paper by Penrose, deal with a particular approach or piece of research.

The Jubilee Celebrations were intended as a survey of the work done in the fifty years from the " rediscovery " and of the perspectives for the future: this volume certainly achieves this purpose well and pleasantly.

G. PONTECORVO

GENES, PLANTS AND PEOPLE. Essays on Genetics. By C. D. Darlington and K. Mather. xxi+187 pp. London: George Allen & Unwin. 16s.

This book is a symposium of the semi-technical writings of two geneticists at successive stages in the development of their thought. At the very least, it provides in compendious and accessible form a number of published papers

which the younger geneticist can now hardly hope to get as reprints; but, fortunately, the writings of both authors show a continuity of scientific purpose that raises this volume above the level of genetical *belles lettres*.

Mather's articles begin with, as they later develop, the theory of mating systems, and the importance of an understanding of mating systems is a theme common to both authors. There is an inclination among teachers of elementary genetics to dismiss the theory of mating systems as a mere enumeration of the bye-laws of heredity. And so, in a sense, it is; for mice mate otherwise than men and obey somewhat different rules of gametic union, though their genes may be supposed to behave in very similar fashion. Elementary expositions of genetics therefore concentrate on those rules (of the behaviour of chromosomes and genes) that are of all but universal competence, and tend to neglect those that govern the coming together of gametes, because they vary from one species to another or even (as with human beings) from one part to another of a single one. The logical wrongheadedness of such a treatment is clear enough: that genes are the coins of lowest denomination in genetical transactions is simply an analytic fiction, for in *fact* gametes are the only legal tender. Chromosomes from two animals will combine only if their gametes do. The theory of the modes of gametic union has therefore some claim to genetical priority.

Mather's later essays deal with " continuous " variation, i.e. with discontinuous variation so fine-grained and so rich in combinational variety as to determine a virtually continuous spectrum of character differences; with genetical buffering systems (cybergenetics?); with the evolutionary import of chromosome behaviour; and with the operational definition of " the gene ". Mather is an analyst by temperament, and it is interesting to see how his essays reveal a chronological increase of analytic power. It is most apparent in his essay on " The Gene ". The " nature of the gene " is a function of the operations used to define it: there is a Mendelian character-difference gene, a gene of mutation, a gene that is the unit of chromosome pairing, and so on. The integrity of genetic theory depends upon their being a high degree of overlap and therefore of interchangeability between these several concepts. Mather's analysis is first-rate, though it would have been enriched by some treatment of cognate problems in other sciences. Physicists have these difficulties too.

Darlington's essays are representative of all his major contributions to genetic and cytological theory: on the relationship between meiosis and mitosis, of which he provided the first complete theory; on chromosome mechanisms considered no less as the products than as the instruments of evolutionary change; and, most far reaching of all, on the relationship between heredity, development, and infection. Darlington's prose style, in his later essays urgent, peremptory and intolerant, seems to reveal an impatience of fine analytic or inferential thought. " If this is not correct," says Churchill of his own exposition of the principles of Radar, " it should be "—and there is something of this intellectual temper here. Darlington's is a mind that moves most easily upstream of the flow of deductive inference —the sort of mind, indeed, that is responsible for formulating the great connecting principles of science. His interpretation of the affinity between the mechanisms of heredity, development and infection reveals an enormous intellectual grasp: no biological synthesis of comparable pretensions has been set before the public in the present century. His hypotheses may be criti-

cised in two sorts of ways. On the one hand, it may be said that the facts he starts with are wrong, or, if right, are made to depend from hypotheses that lead to unfulfilled predictions. It is altogether proper that criticisms of this sort should be made, and so they have been. On the other hand, it may be argued that speculation of this degree of rarefaction is in itself a somewhat disreputable activity, stifled at birth or by early training in those with a better-developed sense of scientific propriety. This is a most mischievous attitude of mind. All sciences, as would-be organised bodies of information, have to counteract the pressure of a sort of intellectual entropy —that is, the dissipation of knowledge into a rabble of particular unrelated facts. Integrative thought of the sort and on the scale indulged in by Darlington is an essential corrective to this tendency; it is all that prevents biology or, indeed, any science, from deteriorating into a mere taxonomy of scientific facts.

P. B. MEDAWAR

SOVIET GENETICS. By Alan G. Morton. London. Lawrence & Wishart. 1951. Pp. 174. 15s.

Dr Morton's book is described on the dust-cover as an unbiased account of the Michurinist theory of heredity with supporting scientific experiments. The author does not claim years of experience in Genetics or plant breeding, and thus he is free from the necessity of believing in any one theory of heredity. He should be able to make a perfectly balanced statement.

Despite the difficulty of penetrating " the curtain of ignorance and misunderstanding with which the Soviet Union is unfortunately so frequently surrounded ", the Michurinist theory is now familiar to most geneticists outside the U.S.S.R. But it will be new to most readers to learn that the regularities of Mendelian ratios—including the precise segregation in the tetrads of pollen grains and reproductive spores of some fungi—are now accepted by Michurinists. Clearly, since 1950, Soviet scientists have benefited from foreign travel. They still deny, however, the existence of determinant particles or genes and explain Mendelian segregation as the " result of the destabilised or shaken heredity caused by hybridisation ".

Turning to the facts on which the Michurinist theory is based we find the true and orthodox Mendelian statement on page 96 that the red tomato fruit is dominant to yellow, and yet on page 101 we find: " Of 633 control fruits in F_1 all were yellow." Has F_1 taken on a new meaning in Michurinist experiments or have the controls been shaken by mistake? Unfortunately it is impossible to decide even after repeated reading. But some experiments are described without such ambiguities. For example there is Khachaturov's (1939) selective fertilisation in Tobacco. First generation hybrids were self-pollinated with amounts of pollen on each pistil varying from five grains to a large mass. The second generation hybrids derived from the large mass of pollen were " rather uniform in height, earliness and appearance ". " The plants from the low pollen fertilisation were much less uniform in character, and half of them were of types not found among the normal F_2." This is a very interesting and important demonstration of the effect of selection on the male gametophyte. Dr Morton concludes: " These data are at variance with accepted Mendelian ideas of the ' purity of the gametes '." If this is the author's personal interpretation he has failed to grasp the elements of Mendelism; if it is an inspired conclusion it has

not been brought into line with the now accepted reality of precise segregation in the tetrad.

D. LEWIS

SCIENTIFIC SESSION ON THE PHYSIOLOGICAL TEACHINGS OF ACADEMICIAN I. P. PAVLOV. Academy of Sciences and Academy of Medical Sciences. 1951. Moscow: Foreign Languages Publishing House. 174 pp. 2s. 6d.

This pamphlet reports in an official translation some of the papers given in a discussion of the present position of Pavlov's teaching in the U.S.S.R. The speakers show that Pavlov's work agrees in all points with the principles of Lenin and Stalin and also of Michurin and Lysenko. They assert that nevertheless much work on nervous physiology is now being carried out by Academician Orbeli and his disciples which disregards Pavlov's principle and " Michurinian biology ", adhering rather to " formal geneticist views ". In reply to these criticisms Orbeli is said to have made a statement admitting " the erroneousness of his first speech ", but failing to " give a lucid criticism and analysis of his errors ". Orbeli's statement is not reported but this was the wording of the resolution of the Academy of Sciences which concluded the session and demanded the reform of all physiological teaching and research in the U.S.S.R. No dismissals were recommended on this occasion but it was resolved to have annual conferences in future to continue the discussion.

C. D. D.

AGROBIOLOGIE. By T. D. LYSSENKO. Arbeiten über Fragen der Genetik, der Züchtung und des Samenbaus. Redaktion der Deutschen Ausgabe. W. Höppner. 1951. Berlin. Verlag Kultur und Fortschritt. Pp. 1-670.

A German translation of the collected essays and addresses of T. D. Lysenko beginning with " The Theoretical Bases of Jarovisation " in 1934 and concluding with " J. W. Stalin and the Michurinite Agrobiology " in 1949. There is a bibliography of the author's hundred most important papers from 1923 to 1947 prepared by I. J. Glushchenko.

SONS AND DAUGHTERS. Roger Pilkington. 1951. London: Allen & Unwin. 214 pp. 18s.

This book is an attempt to introduce to a general but intelligent audience the facts of development and heredity (in that order) as they apply to man. The author's understanding of the fundamentals of the subject is much deeper than his delightfully vivacious manner would suggest to the soberly technical reader. He really believes in genetics although he misses some of its finer points, for example the effects of inbreeding on populations. He spells H. J. Muller's name wrong and he makes Francis Bacon a contemporary of Isaac Newton. His notions that the chromosomes were discovered in the twentieth century is a more serious post-dating. And the reviewer sheds a tear to see two-strand crossing over considered more suitable than four: after all crossing-over, correctly described, is easier to understand than the implantation of the ovum; and it is of greater consequence for life. Otherwise Pilkington's treatment is penetrating and sound and his information abundant and up to date. His 39 photographs are a joy to see.

C. D. D.

THE NATURE OF FOWL NEPHRITIS. W. G. Spector, M.A., M.B., B.Ch., M.R.C.P., A.R.C. Report Series No. 11. 1951. London: H. M. Stationery Office. Pp. 16. 3s. 6d.

The incidence of disease among experimental animals offers opportunities for discovering the genetic basis of variation in disease resistance, opportunities which have been exploited by J. W. Gowen and others in the United States working with men, mice, guinea pigs and above all in poultry. Unfortunately in Britain disease is usually studied by medical and veterinary investigators who have not found it necessary to make themselves acquainted with genetic principles, methods, or discoveries.

Different breeds, and different inbred lines of an unnamed breed, of fowl raised together are found by Mr Spector to vary in the incidence of Fowl Nephritis from 3 to 52 per cent. " None of these differences ", according to his Report, " are statistically significant ". Hence it is concluded that " the results of the investigation do not support a congenital basis for the disease ". The seventeenth century term *congenital*, so much cherished in medical usage, is intended presumably (in this case) to mean *genetic*. Until the numbers are published however we need not assume that the investigation was as unprofitable as it seems.

C. D. D.

MENTAL PRODIGIES. Fred Barlow. 1951. London: Hutchinson Sci. & Tech. Pub. Pp. 256. 12s. 6d.

A history of prodigies in calculation, music, language, and chess, the common factor in all of which is an aptitude for a specialised kind of memory. There are some notes on the heredity of the Bachs and Bernoullis and a great deal of evidence of genetic determination.

GENETICAL SOCIETY OF GREAT BRITAIN

ABSTRACTS of Papers read at THE HUNDRED AND EIGHTH MEETING of the Society held on WEDNESDAY 2nd APRIL 1952, at the UNIVERSITY OF LEEDS

WHAT IS *GERANIUM ANEMONEFOLIUM*?

W. JACKSON
Botany Department, University of Leeds

G. anemonefolium L'Hér. is endemic to Madeira and the Canary Islands. Material was introduced into this country in 1778. Plants collected in Madeira in 1949 were found to have $2n = 128$, whereas the published number for the species is $2n = 68$. This new count prompted the assembling of material from various botanic gardens and commercial firms. These plants exhibit a wide range of morphology and fall into two groups cytologically, those with $2n = 128$ and those with $2n = 68$.

A plant obtained from Kew under the name "*G. canariense*" resembles a gigantic *G. robertianum*. It is an annual and lacks the caudex typical of *anemonefolium* but its chromosome number is $2n = 128$, double that for *G. robertianum* ($2n = 64$).

Nevertheless a culture obtained from the Royal Botanic Garden, Edinburgh, resembling the Madeiran type in many ways, shows 68 chromosomes, while another stock obtained more recently from the same garden belongs to the category with the higher chromosome number.

Intercrossing of the specimens has been started, with limited success. *G. robertianum* is included in the crossing programme. It seems fairly certain that *G. anemonefolium* would be better classified in the section Robertiana Boiss., rather than as the sole species of a section Anemonefolia.

CYTOTAXONOMIC STUDIES IN THE PLUMBAGINACEAE

H. G. BAKER
Botany Department, University of Leeds

In the tribe Staticeae, dimorphism of pollen and stigmata is found and in one section of the genus *Limonium* this appears to be linked with heterostyly. A preliminary survey of the family followed by a detailed survey of *Limonium* on this basis and an experimental taxonomic study of *Armeria* have revealed the necessity of a chromosome-survey of the tribe. This is now in progress. Many species of *Armeria* have been investigated, and so far, all have been found to be diploid ($2n = 18$) including the very widespread *A. maritima* Willd. This is in striking contrast to the remaining genera. In *Limonium* (where previous cytological results have been shown to be quite unreliable) diploids on 6, 7, 8 and 9 have been found, together with tetraploids on the last three basic numbers. $3n$, and aneuploid $3n$ and $4n$ numbers occur in apomictic species. Generally there is good correlation with morphologically based taxonomy. *Goniolimon* and *Acantholimon* contain tetraploid species ($2n = 32$) while *Limoniastrum monopetalum* Boiss. is a very high polyploid.

Apomixis occurs in the sub-sections Densiflorae, Dissitiflorae and Steirocladae. Sexual species are $2n$ or $4n$. *Limonium lychnidifolium* O.K. ($2n = 25$) is believed to have arisen by hybridisation between the sexual *L. ovalifolium*

O.K. ($2n = 16$) and the apomoctic *L. binervosum* C.E.S. ($2n = 35$). The variable *L. binervosum* is being investigated in detail together with the allied " species " which appear to be endemic to the British Isles.

THE PRESENT PROGRAMME OF NEW WORK ON THE CYTOLOGY OF THE PTERIDOPHYTA

I. MANTON
Botany Department, University of Leeds

THE PRESENT STATE OF WORK IN *ASPLENIUM* AND *POLYPODIUM*

M. G. SHIVAS
Botany Department, University of Leeds

THE *DRYOPTERIS SPINULOSA* COMPLEX IN EUROPE AND AMERICA

S. WALKER
Botany Department, University of Leeds

EXPRESSION OF GENES AFFECTING A QUANTITATIVE CHARACTER IN TWO DIFFERENT ENVIRONMENTS

E. C. R. REEVE
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One of the most important problems in animal breeding research is the question of how far a change of environment will alter the effects of the different genes controlling a quantitative character, since this determines the extent to which improvement made by selection in one environment will carry over into a different environment.

Genotype-environment interactions (changes in gene effect with change of environment) have a place in most gene models of quantitative characters, but no attempt seems yet to have been made to measure their relative importance. A simple approach to this problem can be made by an extension of the idea of genetic correlation, if we consider only two contrasted environments, in each of which animals may be reared and measured. This consists in estimating, by means of progeny tests or selection experiments, the correlation between the values which the character would be expected to have in animals of identical genotype reared in the two environments. Such an estimate can be obtained by comparing the four regressions of offspring phenotype on mid-parent phenotype, when each is reared in the two environments, or by an analysis of variance and covariance of progeny means. The method is used in analysing data on body-size in *Drosophila melanogaster* reared at 18° and 25°.

GENETIC ANALYSIS BASED ON MITOTIC CROSSING-OVER IN HETEROZYGOUS DIPLOIDS OF *ASPERGILLUS NIDULANS*

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Strains of *Aspergillus nidulans*, having diploid nuclei in their vegetative cells (hyphae) and teraploid nuclei in the zygotes have been produced by means of Roper's technique (*Experientia*, 1952) and investigated for the occurrence of " mitotic " crossing-over. The results are that: (1) mitotic crossing-over and segregation occur in this species, with varying frequencies in different strains and under different conditions; (2) mitotic crossing-over can be used for mapping the chromosomes, including the location of centromeres; and (3) in *A. nidulans*, the available knowledge—gained *via* standard

genetic analysis based on sexual reproduction—has made it possible to compare the sequences of certain loci, inferred from mitotic crossing-over, with the sequences of the same loci established in the ordinary way and to find agreement between the two. Clearly, however, genetic analysis *via* somatic crossing-over, based as it is on segregation and recombination is valid *per se*. Thus species of this and many other groups of micro-organisms lacking a sexual stage are now open to genetic analysis, provided: (*a*) it is possible to produce heterozygous diploid nuclei, and (*b*) these diploid nuclei undergo mitotic crossing-over. As regards (*b*), the high frequency of mitotic crossing-over in certain cases in *A. nidulans* and its high sensitivity to as yet unanalysed external and internal conditions suggest that crossing-over is a more general and fundamental feature of chromosome duplication than was hitherto suspected. In a species like *Aspergillus*, not normally diploid, mitotic crossing-over occurs frequently when a chromosome is provided with a partner with which to cross over. Perhaps the evolution of the diploid stage in groups where it now extends over most of the life cycle has required side by side the evolution of mechanisms to reduce crossing-over in mitotic cells and confine it to meiosis in the germ cells.

A preliminary analysis has been carried out of three diploid strains, each heterozygous in varying combinations for three out of five linked genes (*w*—26—ad_1—21—ad_2—7—*y*—5—*bi*, in this order) and other non-linked genes. Further tests of recombination in recombinants for certain markers suggest, as expected, that homozygosis for one locus goes with homozygosis for the loci distal to it but not necessarily for those proximal, or on a different arm, or on a different chromosome. Mitotic crossing-over does not seem to occur at random among all nuclei, but rather to be concentrated in certain nuclei. The technique is obviously applicable to the controlled " breeding " of asexual organisms of industrial importance.

THE INFLUENCE OF MINUTES UPON SOMATIC CROSSING-OVER IN *DROSOPHILA*

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Four second chromosome Minutes, two located on the left arm and two located on the right, were chosen to study the Minute influence upon somatic crossing-over. It was demonstrated that the Minutes increase the frequency of crossing-over above the control rate. In general the influence is restricted to the arm of the chromosome in which the Minute is located. However, a left arm Minute, M(2)S5, located within or close to the heterochromatic block adjacent to the kinetochore influences the right and left arms of the second chromosome non-preferentially. Moreover M(2)S5 and M(2)Z, the second left arm Minute, influence the second chromosome differentially, the region within which crossovers occur most frequently differing in the two cases.

Heat treatment significantly increases the crossover frequency above the rate at 25°C. In the presence of Minutes and high temperature some crossovers occur that would not have taken place under either single condition.

A sex-linked Minute significantly increased the rate of crossing-over of the second chromosome control rate, and the sex chromosome in turn was influenced by the autosomal Minutes. Heat treatment decreased the frequency of X chromosome crossing-over in the presence of the sex-linked Minute.

CORRECTION—Vol. 6, Part 1

On page 121, paragraph 5, line 2, "*aa*" should be "*A*-".

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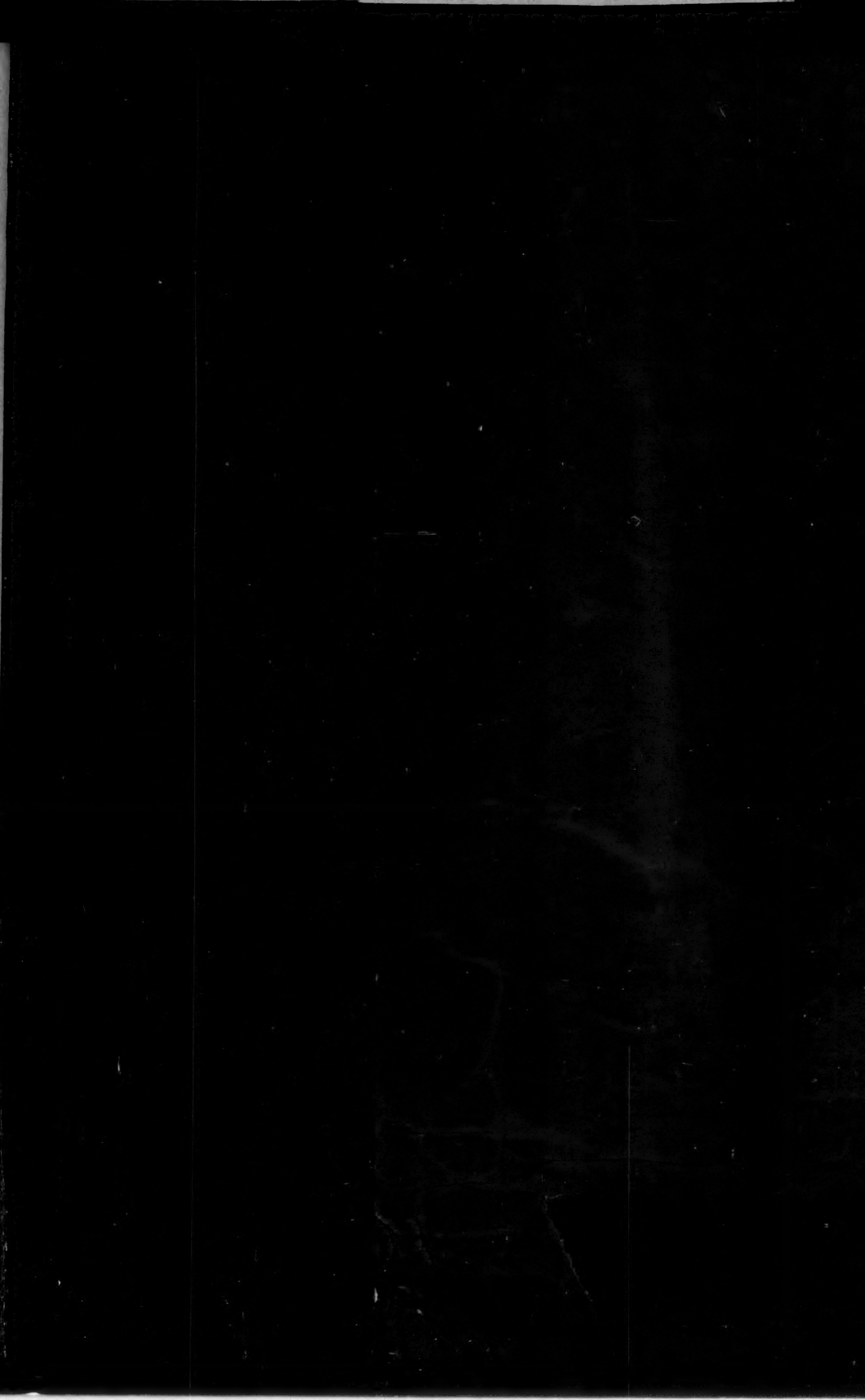
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